VOLUME 32 SEPTEMBER 1954 NUMBER 5

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF BOTANY

(Formerly Section C, Canadian Journal of Research)

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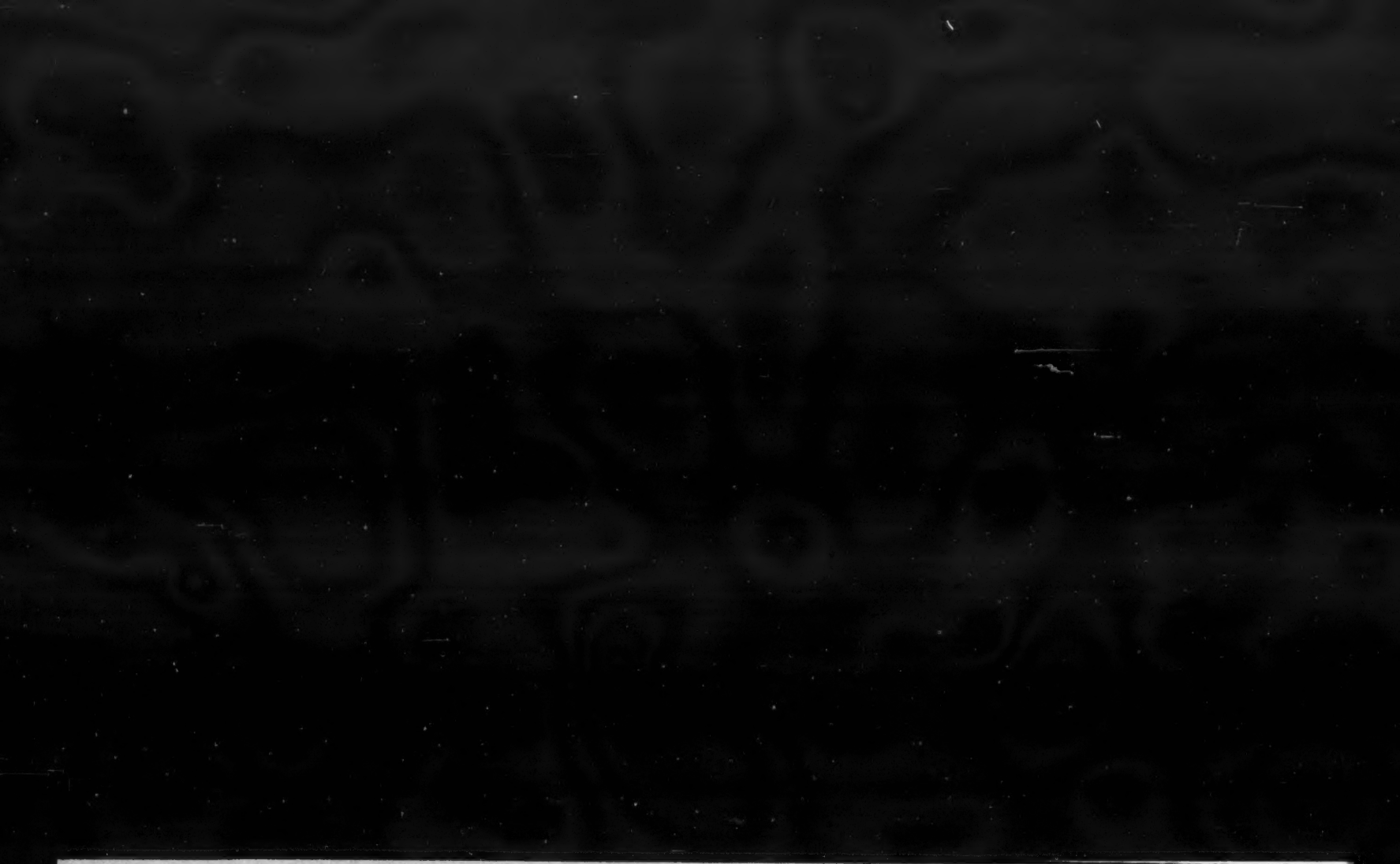

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VOLUME 32 SEPTEMBER 1954 NUMBER 5

BOG AND LAKE STUDIES ON THE LAURENTIAN SHIELD IN MONT TREMBLANT PARK, QUEBEC[1]

BY J. E. POTZGER[2] AND ALBERT COURTEMANCHE[3]

Abstract

The study includes five bogs and one small lake. Preferred kettletype bogs were located very effectively by plane. The deepest section of the kettle lake was determined with a Bendix Depth Finder. The initial tree invaders in the whole park were chiefly jack pine (*Pinus banksiana*), paper birch (*Betula papyrifera*), a minor representation of spruce–fir (*Picea–Abies*), and oak (*Quercus*) characterized by an attention-demanding minor peak. After one or two foot-levels spruce–fir increased to considerable prominence, then declined to negligible representation in all bogs and in the lake by mid-profile. The pine peak at about mid-profile was uniformly very prominent over the whole area. In the topmost one-third to one-fifth of the profiles, pine declined steadily. The small representation of pine at topmost levels pictures very well its status in the region today. Mont Tremblant Park is a forest transition zone. In the range of 50 miles from Lac Puant bog to Lac Tacoma bog, abundance of spruce and fir pollens (especially that of black spruce) increase markedly as one proceeds northward. Pollens of beech (*Fagus*), oak, maple (*Acer*), and especially hemlock (*Tsuga*) and yellow birch (*Betula lutea*) show marked decrease northward. In the southern half of the transect hemlock is represented in the profiles by a decided pine–hemlock peak. Paper birch has erratic fluctuations in its prominent representation over the whole profile. From the forest type indicated at lowest levels we must infer an initial warm period. This is attributed to ocean invasion of the St. Lawrence Valley and slow retreat of continental glaciers. The combination of these factors delayed the forest invasion of the Canadian Shield until the boreal spruce–fir period, so characteristic of lowest levels in bogs farther south, had passed. Slight deterioration of climate thereafter with development of local glaciers on the Shield apparently accounts for the first minor rise of spruce and fir. A warm–dry period at mid-profiles is indicated by the universal prominent pine peak accompanied by extreme decline of spruce and fir. The succeeding increase in hemlock and white–red pine (later replaced by minor peaks of beech–maple) suggests a moister climate with increasing coolness. Continued cooling during more recent times in turn favored spruce, fir, and yellow birch. Pine, hemlock, beech, and oak declined to a status of minor elements in the forest association. The erratic fluctuations of paper birch and its abundance at all levels show little correlation with major climatic changes.

[1] *Manuscript received March 17, 1954.*

This is contribution 272 from the Botanical Laboratories of Butler University, and contribution 11 of the Service de Biogéographie, Université de Montréal. We express our thanks to Mr. Robert Lipscomb for assistance in the field and with laboratory work; to Mrs. Margaret Esther Potzger for preparation of slides and preliminary graphs; to Mr. Florian Grenier for surface samples from Lac aux Atocas, taken with his sampler; to the Mont Tremblant Biological Station of the Game and Fisheries Department, Province of Quebec, for laboratory facilities and all-round helpfulness in field work, especially in providing aircraft for locating bogs, as well as rowboats and the depth finder used in lake work; to district officers and foresters of the Canadian International Paper Co. and Consolidated Paper Corp. for their generous help, courteous hospitality, and valuable information. We are especially indebted to Mr. J.-Emile Fortier, chief scaler of the Canadian International Paper Co. at Saint-Jovite, for the excellent summary on lumber operations in the Mont Tremblant area.

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[The July number of this Journal (Can. J. Botany, 32 : 503-548. 1954) was issued June 30, 1954.]

Introduction

In a land area as vast as Quebec, with its maze of physiographic and climatic variants, and especially microclimatic differences, one would naturally expect a complex pattern of vegetation types. Even a casual study will prove such an assumption to be correct. The differences just referred to are especially emphasized from the Gaspé into the St. Lawrence Lowland, but are not absent on the Laurentian Shield. Halliday (6) and Halliday and Brown (7) reflect the complex habitats in their vegetation maps. In a recent pollen study of bogs in southern Quebec, the senior author (12) recognized the same characteristics as a feature of the postglacial history of the forests.

The present study (Fig. 1) is a continuation of the pollen study just referred to. It aims to bring the Laurentian Shield into the picture of the postglacial forest history of lower Quebec.

Geographic and Geologic Features

Mont Tremblant Park is a section of the southern slope of the Laurentian Highland about 100 miles north of Montreal. It covers an area of 1204 square miles (Canada Yearbook, 1948–49). The rocks are primarily Pre-Cambrian granites. During Pleistocene time the region was covered by the Keewatin icecap. Effects of glacial activity are evident in fjordlike valleys, boxlike walls of small canyons, and gouged-out rock basins now filled by lakes.

Forests

According to Halliday (6) the region belongs to his L.4 and B.7 divisions. Of L.4 he says: "This quite extensive section (Algonquin-Laurentides Section) takes in the Georgian Bay district. . . . In this Section, white pine probably reached its maximum development in Canada, but extensive lumbering and fire have removed the greater part. Red pine has also been a prominent species, especially on the Algonquin Highlands. In spite of the previous dominance of these species and the presence of intrusive conifers from the Boreal Forest Region, the general character is that of a mixed forest, and the dominant or competitive association is one of sugar maple, yellow birch, hemlock, and white pine. . . . The composition of this association changes somewhat to the north, as hemlock (Northern) red oak, and beech decrease numerically and finally drop out before the limits of the Section are reached and the proportion of yellow birch, white spruce, balsam fir, and white birch increases. White pine was originally of much greater representation in this association. . . ."

The southern part of the park grades into Halliday's Section B.7. He says: "The general character of the forest is a mixed one, consisting of an association of black spruce, balsam fir, and white birch, with scattered white spruce and aspen. This appears the dominant and mature type on the middle slopes. Here and there will be found, on heavier-textured ridges, patches and specimens of both sugar maple and yellow birch—more commonly in the Quebec

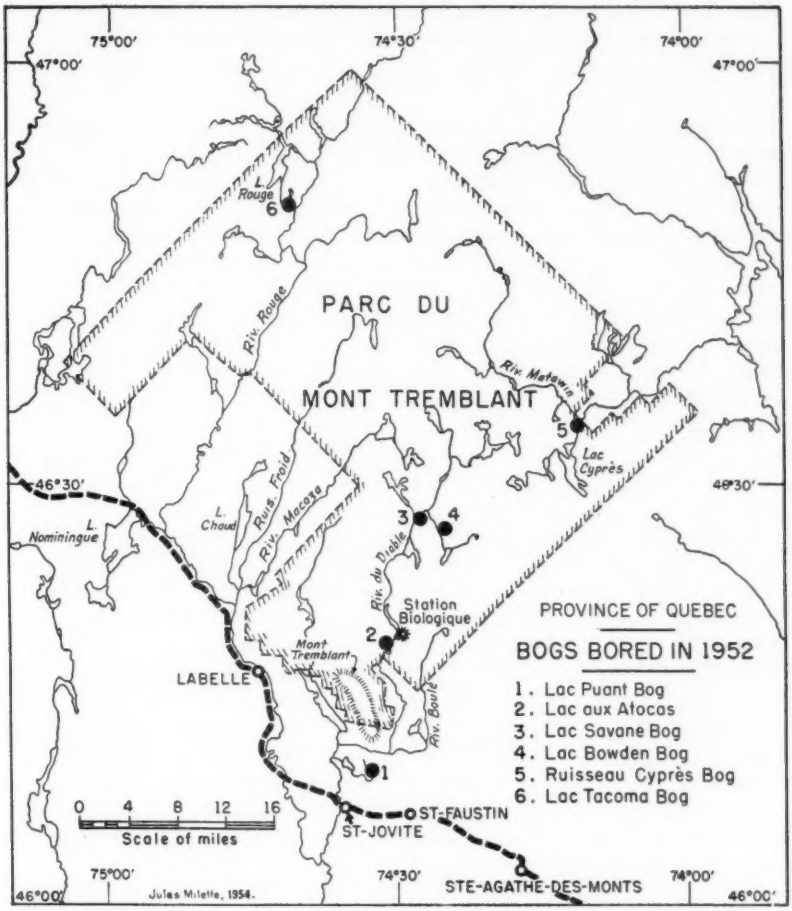

FIG. 1. Outline map of the Mont Tremblant area and location of bogs.

portion. On rocky shores, and also on ridges, there is some presence of white and red pine. . . . Jack pine occupies dry ridges and sandy flats. . . ."

One can easily infer from the previous descriptions that the forest is a tantalizing mixture of boreal and broadleaf species which is not easily explained or understood. At times spruce and fir constitute pure stands; again, yellow birch may predominate, especially on slopes and tops of low mountains. In the Lake Monroe region yellow birch no doubt dominates the forest cover as a whole, while in the Lac Cyprès and Lac Rouge area spruce, fir, or locally jack pine, play the most prominent role. Pine and hemlock are only minor

elements in the forests. Soil and topography are very likely the chief controlling factors in the heterogeneous distribution pattern of forest cover types.

Methods

The rugged topography at first seemed a difficult barrier to the locating of suitable bogs of the kettle type, but the plan of the junior author to use an aircraft for reconnoitering proved a most successful and excellent solution to the problem. The nature of the bog can thus be ascertained much more efficiently than one can ever hope to do by mere surface observation. The character of the basin, the ramifications of the adjacent valleys, location with reference to streams or chains of kettles, all could be determined at a glance. The location of suitable bogs with reference to roads and nearby lakes was indicated on a map, and they could then be reached without great loss of time. In some instances bogs were very close to roads but were hidden behind dense bordering forests.

Borings were made with a 17-in. Hiller-type borer, taking a 1.75-in.-wide core. At least one foot of sand was included in each core. At times the sandy layer had pollen content, at other times it had none. Small samples of the peat were placed into 16-ml. vials which were stoppered securely. Lac aux Atocas is a small kettle lake. The boring here was also made with the Hiller borer through 20 ft. of water. The deepest part of the kettle was located with the Bendix Depth Finder, and boring operations were conducted from two rowboats securely lashed together and firmly anchored. This gave support for two workers to exert sufficient pressure on the rods to drive the borer into the densely compacted lower sediments. Since the customary peat sampler cannot take samples in dilute uppermost sediments of lakes, the Grenier sampler was used to secure very satisfactory samples from the upper 6 in. of sediments. This section of the core should present forest history during the last 75 years when civilized man became a significant biotic influence. All bogs, except the Lac Puant bog and the Lac Tacoma bog, were of small kettle type in which initial exploratory borings with a point bit easily determined the deepest part of the basin.

In the laboratory the Geisler (5) alcohol method was used to prepare slides because all the peat separated easily. Gentian violet proved again to be an excellent stain which made pollen grains so conspicuous on the slide that tabulation was greatly facilitated. Counts of 150 to 200 pollens of trees were made at all foot-levels.

Results

Graphs 1–6 of Figs. 2 and 3 present results in summary form, but it seems desirable to draw attention to some of the most important highlights of the forest history. As pointed out by Halliday (6), the forest in the Mont Tremblant region is of the transition type. This was a characteristic during most of its postglacial history. Here northward extension brings temperature into play as a vital control factor. This is emphasized by the northward

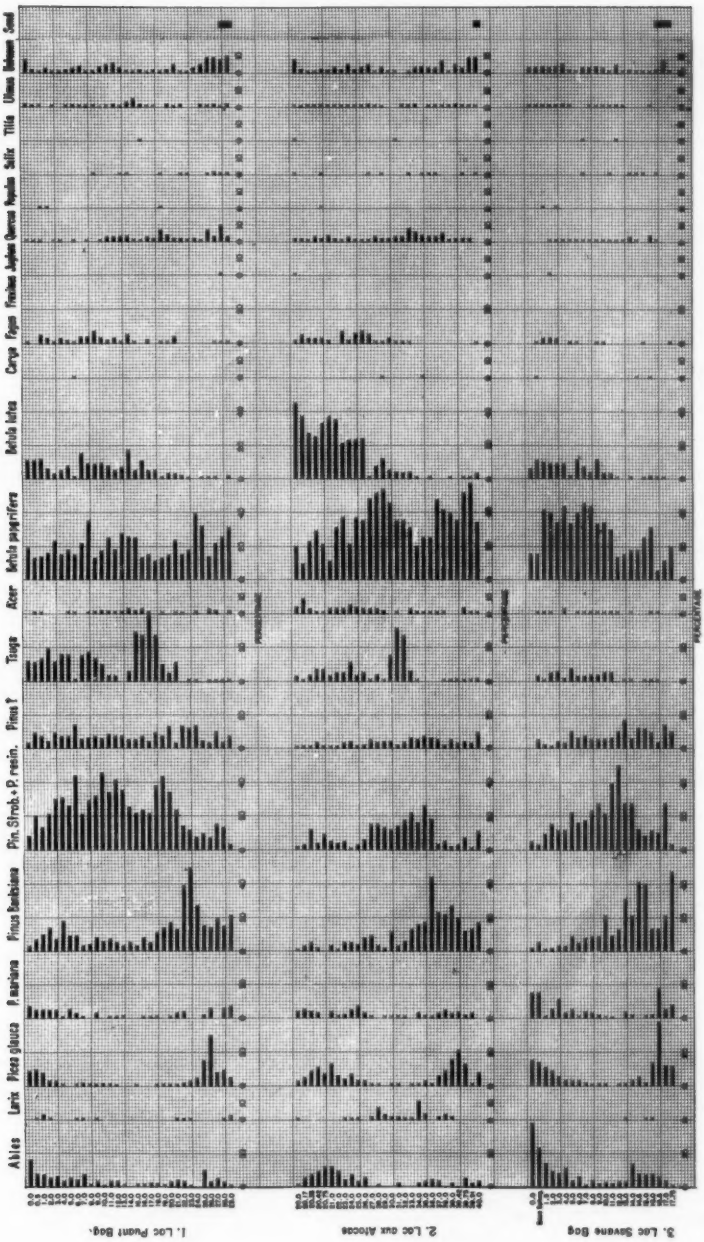

FIG. 2. Profiles of Bogs 1, 2, 3.

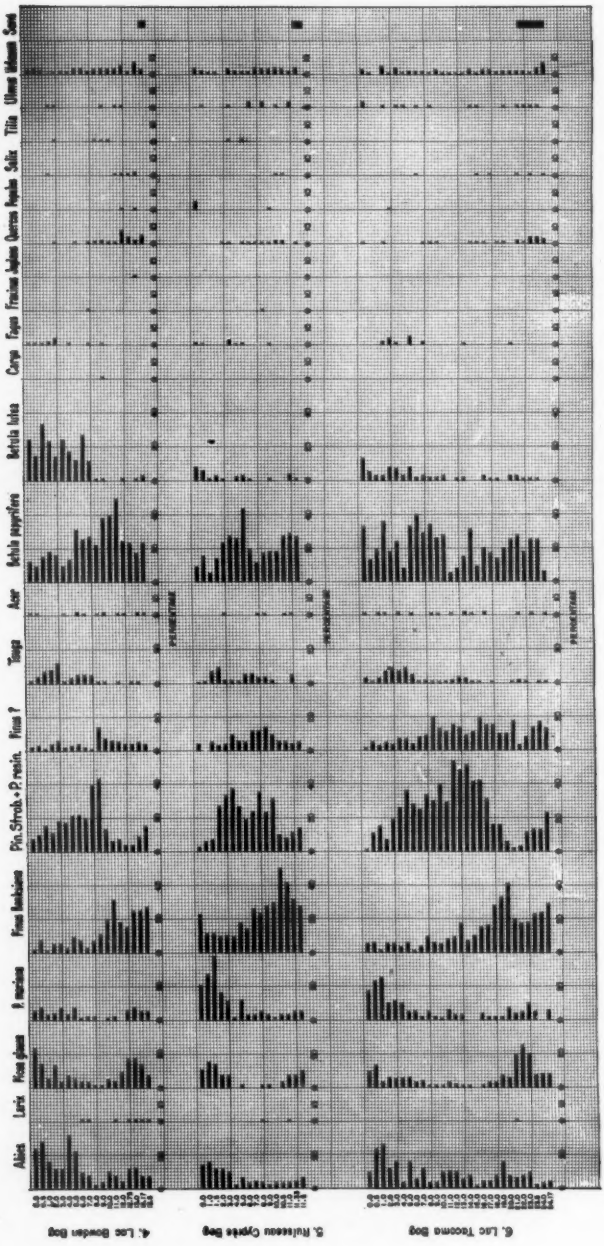

FIG. 3. Profiles of Bogs 4, 5, 6.

decline of hemlock, yellow birch, beech, and maple (throughout the whole history as well as today). The latitude of Lake Monroe is approximately the northernmost point where hemlock gave evidence of important crown control in the forest cover (Fig. 2, Bog 2). While the hemlock peak was evident northward of this point, the degree of the peak became progressively reduced. This "tension zone" position added range limit potentialities to this study of forest history.

The initial forest history approximates that presented by the profiles of the 19 bogs from southern Quebec on which Potzger (12) reported recently, i.e. an initial warm period was characterized there as here by jack pine, paper birch, and an attention-demanding minor peak of oak. A colder period followed, expressed by increased prominence of spruce and fir. About mid-profile pine reached its maximum and spruce and fir dropped to a minor position. Jack pine very evidently constituted the early part of the pine peak, for the pollen grains at this point were all very small. The latter half of the peak was due chiefly to increase in white–red pine. During this period hemlock came into great prominence as far north as Lake Monroe. Synchronous decline of pine and hemlock was accompanied by invasion of such northern hardwoods genera as beech, maple, and yellow birch, especially the last. While this forest association had its best development up to the latitude of Lac aux Atocas, it is still evident northward to Lac Tacoma, the northernmost station included in the present study. It appears that cooling climate, perhaps also more moist climate, at first favored the northern hardwoods, but continued deterioration favored more and more the boreal spruce and fir element, as the bimodal peak of spruce and fir well indicates (Figs. 2 and 3).

Pine never recovered from the decline which began shortly after the mid-profile high; it gradually and consistently lost ground to the very present, except at Lac Cyprès, where jack pine and black spruce became aggressive during recent times (Fig. 3, Bog 5). No doubt this difference in the regional picture is due to microclimatic factors, such as those associated with sandy soil. The jack pine stands near the lake were cut within the past few years, some last year, and we climbed over the slash during the field trips in that region.

Paper birch in Quebec has always defied an adequate explanation for its erratic peaks and troughs, and there is no change in this problem in the forest history of the Mont Tremblant Park.

The only profile where one can be certain that the forest history of the past 75 years has been recorded is in the upper 6 in. of soft sediment from Lac aux Atocas (Fig. 2, Bog 2). Although 6-in. and surface levels of Lac Savane, Lac Bowden, and Lac Tacoma bogs picture the same progress of forest history, we do not read their closing records with such confidence as we read those of topmost sediments of lakes. The one-foot level of Lac aux Atocas clearly shows the waning pine period, we know that at this time civilized man came with saw and axe to begin lumber operations. He laid low the isolated stands of pine; yellow birch invaded the open areas and pine

dropped to relic status. This is very obvious in Mont Tremblant Park today; one must look closely for pine in the forest association.

One is greatly impressed with the similarity of the forest history pattern in all the bogs studied. This suggests that the controls were of regional magnitude.

Discussion

The forest history of Quebec is almost incomprehensible without a knowledge of the complex glacial history of the region. South of the St. Lawrence valley, retreat of the ice was more or less the single controlling factor of forest migrations. Southward of Quebec there were apparently no major subsidiary climatic fluctuations to make significant changes in the forest composition. In Quebec the wasting icecap was no doubt retarded in its retreat by the more or less local effects of the higher elevations of the Laurentian Upland, as Flint (3) also postulates. The St. Lawrence valley constituted an extensive sea which submerged not only the lowland levels but even the flanking southern slopes of the Highland up to 700 ft. elevation.

The geologic and climatic history of Quebec is complicated further by intrusions from local glaciers on the Shickshock Mountains, the Laurentide Park region, and perhaps in other localities. According to recent discoveries by Osborne (8) these local glaciers had developed after disappearance of the Laurentide icecap, and sent tongues of ice into the St. Lawrence Lowland. The same author suggests that a similar local icecap might have developed on the Morin monadnock, within the region where the present pollen study was made. If the xerothermic theory of Sears (16) is correct, we might well assume that the warming climate quickly terminated the remnants of the Keewatin and the Laurentide icecaps and thus made Quebec ready for forest invasion. In such case the usual extreme boreal climate was wanting, eliminating thus a pronounced early spruce–fir period. The prominent minor peak of oak in lower foot-levels and prominent peaks of jack pine and paper birch lend themselves to such assumption.

Delayed invasion by vegetation, determined more or less by local conditions, and moderating climate would also rule out a tundra, at least on the lower slopes of the Laurentian Shield. None of the six profiles from the basins examined (Figs. 2 and 3) showed even a grass and sedge representation of sufficient magnitude to warrant assumption of tundra conditions when the region emerged from the ice. It should be pointed out specifically that this also applies to the deep Lac aux Atocas (Fig. 1, Bog 2) where certainly no delayed paludification could be assumed as origin of the lake.

Since the very earliest forest period (spruce–fir), usually associated with retreating borders of ice and unobstructed entrance to bare soil, was, apparently, inhibited in the Mont Tremblant region, we deal with a truncation of the potential complete forest history, or, we may say, local geological phenomena made regional climate go unrecorded in the available forest history (Figs. 2 and 3). In this respect the Mont Tremblant area is similar to the pollen records from Isle Royale in Lake Superior (11) where the waters

of Algonquin IV or of Nipissing stages (or both) prevented accumulation of pollen to represent forest history, hence climatic changes also passed unrecorded until forests could invade and leave records in accumulating peat.

A very significant contribution to postglacial history in lower Quebec is the delayed spruce–fir peak (Figs. 2 and 3). It no doubt records the effects of local glaciers where higher elevations of the Laurentian mountains intensified deterioration of regional climate which was not of sufficient magnitude to affect all of eastern North America. It must have come as an intrusion into the greater xerothermic period to which it again yielded after a short time interval. This is indicated by the subsequent sparse spruce–fir component of the forest cover, the prominence of pine and at times also of paper birch, and the persistence of minor oak peaks. Beyond this interlude we find in the Mont Tremblant region a fair correlation with the forest succession of Maine, as shown by Deevey (2) and by Potzger and Friesner (15). The warm–dry period was followed by gradual cooling and improved moisture conditions. This we may read into the hemlock peak, the beech–maple intrusion, and the subsequent more pronounced deteriorating climate of recent times which favored the present forest association of spruce, fir, yellow birch, and a small representation of pine (Figs. 2 and 3).

The "plight" of pines is indicated not only in pollen profiles but is also given emphasis in reports of foresters on happenings in the Mont Tremblant Park region since cultural influence vitalized pressures of succession which the decadent pine could not meet by adequate reproduction. In a private communication from Mr. J.-Emile Fortier, Canadian International Paper Co.'s chief scaler for the district, we learned that: "In the Lake Monroe area (about 1892) pine was cut by choice, about 30,000 board feet annually. For a period of four years the logs removed were 75% pine and 25% spruce. During the following ten years it was 90% spruce and only 10% pine. After 1906, for a period of 35 years percentages were 95% spruce and 5% pine." Today pines are seen only as scattered individuals. This steady decline of pine is also pointed out by Halliday (6) but above all we find it recorded in the upper 5 in. of sediments from Lac aux Atocas (Fig. 2, Bog 2), collected with the Grenier sampler. The 5 in. very likely represent accumulation during the past 75 years, thus recording forest changes during and after lumbering activity in the region.

Recently Dansereau (1) attempted to loosen the "Gordian knot" of the great pine period which is so prominent in all pollen profiles of eastern North America, i.e. to explain whether the pine period is indicative of a specific climate or whether it represents a subclimax condition to broadleaved forest. He includes in his assumptions the possibility of decided genetic changes from protypes to present day northern pines. We readily admit ecotypes in a population of the same phenotypic population but find it difficult to assume great genetic changes involved in the pine period of Quebec. The forest succession there very likely took place within a comparatively short period of time.

There is no doubt that pine represents a "critical" borderline type of climate, fitted between spruce–fir and broadleaved forest, but we hestitate to consider it "climatic instability", as defined by Danserеau, for the pine period stands out boldly as a very stable vegetation type, not harassed by short interval fluctuations due to competition from spruce–fir on the one side and broadleaved on the other. Of course, profiles from bogs north of the Great Lakes, along eastern coastal regions, and especially from Quebec (Figs. 2 and 3) show a prominent increase in spruce–fir and decline of pine in the upper one-fifth of the profiles, but this is no doubt due to a deteriorating climate in the more recent past.

Pine very likely represents a climate where extremes rather than average exerted the dominating control as Potzger (10) attempted to show for the tension zone of Lower Michigan. If the critical zone of climate was wanting, as it must have been in Tazewell areas of Indiana, the prominent pine period did not enter into the climatic successions but oak more or less immediately succeeded spruce–fir as Potzger (9) has described in some detail. Potzger and Courtemanche (14) found that the red–white pine group once had great prominence on the northern slope of the Laurentian Mountains but they were replaced by jack pine and black spruce. It appears that recent paludification may have been the real cause of southward depression of these pines. In that case the region may even today be potential pine climate.

It is a characteristic of genera hard pressed by macroclimatic changes to maintain themselves in habitats where microclimatic conditions favor them but not aggressive invaders. Friesner and Potzger (4) have shown this for the relic colonies of white pine and hemlock deep within the southern broadleaved forest of Indiana. Habitats where microclimatic conditions differ greatly from the macroclimatic factors (involving perhaps temperature, humidity, insolation intensity, and other factors) may at times be very extensive areas as the sandy plains of the upper Lakes States which, as Potzger (9) pointed out, perpetuated pine forests and, after destruction of the virgin stands by lumbering and fire, maintained the areas as potential "pineries".

In the Mont Tremblant Park area the sandy soil at Lac Cyprès (Fig. 2, Bog 5) is apparently characterized by a microclimate favorable to perpetuation of pine (jack pine) to the present, and this in a setting of macroclimate which is characterized by consistent decline of pine for perhaps the past few thousand years. Pollen profiles show such local differences most excellently (Fig. 2, Bog 5). Last summer we also learned of a smaller microclimatic niche developed by a sandy bar separating two small lakes where a colony of jack pine maintains itself against the pressures of a macroclimatically favored broadleaved forest.

We are no doubt justified to record here some of the known facts about the forest adjacent to and in Mont Tremblant Park when civilized man first invaded the forest primeval. This is in addition to what was said previously about the Lake Monroe area. One is impressed with the mosaic distribution

pattern of such genera as pine, hemlock, and oak, which show such great decline in the pollen profiles. The information came from Mr. J.-Emile Fortier, a man who has long been associated with lumber companies of that region. He bases his report on recollections of an old woodsman, who was a member of the first logging crew which began operations along the Rivière du Diable and continued in the employ of lumber companies until a few years ago, as well as on his own experiences of many years in lumbering activity. He supports the reports with figures from records of lumber companies on kind and amount of timber cut. He says: "Along the Rivière Rouge, lumbering of pine by Moore and Ward (about 175,000 to 200,000 board feet annually) began about 1872 at the mouth of this stream, and in the course of 30 years they extended their operations northward into the valley of the Rivière du Diable toward Saint-Jovite and Saint-Faustin as well as along the Rivière Rouge to La Conception, Labelle, L'Annonciation, Nominingue, Sainte-Véronique and L'Ascension." Other lumber companies then continued for 30 years to remove 18 to 20 million bd. ft. annually, 95% spruce and 5% pine.

Even percentage figures may be misleading when selective cutting prevailed, but all reports make clear that after 30 to 50 years of lumbering the emphasis always shifts from pine to spruce and fir and in some cases to hardwoods, as at present in the Lake Monroe area.

It is extremely interesting to find that the decadence of pine, hemlock, and oak so well marked in the pollen profiles found expression in the forest formation in a mosaic of discontinuous distribution, where microclimate selected certain genera. To quote a few striking examples mentioned by Mr. J.-E. Fortier: "The region about the confluence of the Rivière Boulé and Rivière du Diable was all pine. . . . As late as 1937, between Lac Chaud and the Rivière Rouge, there was a beautiful stand of oak. . . . Hemlock was always present along the lower course of the Rivière Macaza but did not extend beyond Lac Sapin. . . . Hemlock was found at Saint-Rémi, Vendée, La Conception, L'Ascension, between Lac Chaud and Dix-Mille up the Rivière Rouge, but not beyond. . . . Hemlock was found in small amount along the Rivière Boulé, around Lac Français and on the lower slopes of Mt. Tremblant." He further states that hemlock was favored by pine lumbering and became more abundant until recent cuttings.

The authors went to great pains to make sure that bogs were selected very carefully, and much time was devoted to locating the deepest part of the basin. It is, indeed, of great significance to a complete profile that the boring be made in the deepest part of the basin. Potzger (10) pointed out specifically that borings made at the edge of a central pool always yield profiles truncated at lower levels. Potzger and Courtmanche (14) will present further evidence of this same defect in a subsequent paper. We emphasize this error in sampling to warn especially workers new in the field that borings made at the borders of even small pools will not yield a complete forest history, and the literature of today should be spared these unsatisfactory records.

In conclusion it might well be pointed out that a climate transition is especially significant in a forest history of an extensive continental area like Quebec because it presents a broader view of climatic controls. In this respect the Mont Tremblant region is ideally located and makes an important contribution to the total forest history of Quebec. Here certain features of the forest history are still very similar to those in the St. Lawrence valley, as recently described by Potzger (12). This points to similarity in major climatic and geologic changes (Champlain Sea, local glaciers) but also emphasizes modifications in degree of control induced by latitude or altitude, or perhaps by both.

It will be the prerogative of a future study of bogs on the crest and down the northern slope of the Shield to discover if minor changes in climate, still recorded in pollen profiles from the Mont Tremblant region, made their influence felt beyond the southern slope of the Canadian Upland.

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STUDIES IN PLANT MINERAL NUTRITION

II. THE ABSORPTION OF IRON AND MANGANESE BY DWARF KIDNEY BEAN, TOMATO, AND ONION FROM CULTURE SOLUTIONS[1]

By W. Leach[2] and C. D. Taper[3]

Abstract

Dwarf kidney beans and tomatoes were grown in complete nutrient solutions containing various concentrations of iron and manganese. It was found that the iron to manganese ratio in the culture solutions must be within a definite range in order to avoid deficiency symptoms in the experimental plants. For the dwarf bean this range was 1.5 to 3.0, and for the tomato it was 0.5 to 5.0. Concentration ratios outside the optimum range produced either iron or manganese deficiency symptoms. Concentrations below minimum values of both iron and manganese always produced deficiency symptoms irrespective of the ratio of iron to manganese in the culture solutions. An apparent antagonism between the absorption of iron and of manganese was observed with both beans and tomatoes grown in nutrient solutions. This antagonism was also found to occur with onions grown in solutions containing only the sulphates of the two metals. It was further found that with onions growing in single salt solutions of the sulphates of iron and manganese, the concentration of iron or manganese in the solutions did not affect the amounts of the metals absorbed by the plants from the solutions. The combined amount of iron plus manganese absorbed appeared to remain constant irrespective of the iron to manganese ratio in the culture solution.

Introduction

In the present paper there are described a number of experiments designed to investigate the physiological relationship between iron and manganese in certain experimental plants, and also the incidence of chlorotic leaf conditions resulting from deficiencies of these two metals.

A considerable amount of research has been carried out by other workers in this field and the related publications have been adequately reviewed by Shive (6), Stiles (9), and Twyman (10). A number of these researches, however, should receive special consideration in view of their particular significance in relation to the work here described.

In 1942 Somers and Shive (8) revived interest in the long recognized connection that appears to exist between the functions of iron and manganese in plant metabolism. They advanced the theory that the functional iron in plant cells is present in the soluble ferrous state, and that this iron may be oxidized by manganese to the inactive and relatively insoluble ferric form. They found in experiments with soybean that, for healthy development, the ratio of soluble iron to soluble manganese in the leaf tissues must lie between 1.5 and 2.5. Any departure from this ratio range resulted respectively in either iron deficiency or manganese deficiency symptoms. They further suggested that iron deficiency is the same as manganese toxicity and manganese deficiency the same as iron toxicity.

[1] *Manuscript received April 5, 1954.*
Contribution from the Department of Botany, University of Manitoba, Winnipeg, Man. With financial assistance from the National Research Council of Canada.
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Hewitt (1, 2) carried out an extensive series of experiments on sugar beet, oat, tomato, potato, and kale, in which he observed the effects of excess of a considerable number of metals, including manganese, on the incidence of symptoms of iron deficiency. The results of these experiments were not in accordance with the simple oxidation–reduction hypothesis put forward by Shive and his associates. Hewitt further showed that symptoms of both iron deficiency and manganese deficiency could be produced simultaneously on the same plant, thus indicating clearly that a deficiency of one of these metals cannot correspond to a toxicity of the other. Before we finally accept hypotheses based on the oxidation–reduction potentials of simple ions, however, Hewitt holds the opinion that we need to know more about the organic complexes which exist in plant cells and in which metallic elements are involved. When this knowledge becomes available it may well be that the oxidation–reduction concept of iron–manganese relations will prove to be the correct one. Further, it is of some significance that a number of investigators, including Twyman (11), have produced evidence of the presence of active and inactive forms of iron in plant cells.

It is obvious that the present state of our knowledge is inadequate to enable definite conclusions to be arrived at concerning the physiological relationship of iron and manganese in plants. It was for the purpose of furnishing additional data that the experimental work here described was undertaken.

Materials and Methods

The plants used in the experiments here described were dwarf kidney bean, *Phaseolus vulgaris* var. *humilis;* tomato, *Lycopersicum esculentum* (Vetomold 121); and onion, *Allium cepa.*

The analytical procedures and culture methods used, except in onion experiments (see p. 568), were similar to those already described in the previous paper of this series (5). In the onion experiments the culture vessels were supported on a tray in a constant temperature water bath so that they were partially immersed in the water. The tray was mechanically agitated horizontally in order to keep the culture solutions continually circulating around the roots of the experimental plants.

Analyses for soluble iron and manganese in plant material were carried out on acetone extracts as described by Sideris and Young (7).

Culture Solutions

The procedures used in making up culture solutions containing major and minor elements were the same as already described (5).

One nutrient solution was used in all the experiments and its composition is given in Tables I, II, and III.

Manganese was supplied as inorganic sulphate or combined in a potassium humate solution (3). Iron was similarly added to the culture solutions as ferrous sulphate, organically as humate (3), or as chelated iron prepared as described by Jacobson (4).

TABLE I

COMPOSITION OF CULTURE SOLUTION

Salt	ml. 0.5 *M* stock solution per liter
Calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$	9.00
Dibasic potassium phosphate, K_2HPO_4	1.16
Magnesium sulphate, $MgSO_4 \cdot 7H_2O$	4.60
Potassium sulphate, K_2SO_4	1.63
Minor elements (See Table II)	1.00

TABLE II

MINOR ELEMENT STOCK SOLUTION

Salt	gm. per liter of distilled water
Boric acid, H_3BO_3	2.860
Zinc sulphate, $ZnSO_4 \cdot 7H_2O$	0.220
Copper sulphate, $CuSO_4 \cdot 5H_2O$	0.080
Molybdenum oxide, MoO_3	0.075

TABLE III

ELEMENT CONCENTRATION IN CULTURE SOLUTION

Element	N	P	K	Ca	Mg	S	B	Zn	Cu	Mo
Conc., p.p.m.	126.00	18.00	109.20	180.00	55.20	94.80	0.50	0.05	0.02	0.05*

Experimental Results

In the first place a number of cultures were set up and carried on for several weeks for the purpose of determining the characteristic symptoms of iron and manganese deficiencies as shown by the particular experimental varieties used. This was done by growing the plants in culture solutions containing all the mineral nutrients with the exception of either iron or manganese.

In the case of the bean, the early indication of iron deficiency was a uniform yellowing of the youngest leaves. The chlorosis gradually spread downwards to the older leaves while new leaves developing from the apical bud appeared

to be completely devoid of chlorophyll and were almost white in color. No abnormal symptoms appeared in the roots, but the growth of the plants as a whole was definitely retarded.

Manganese deficiency in the bean made its first appearance in the older basal leaves. In these chlorosis appeared, not uniformly as in iron deficiency, but as patches between the veins, giving the leaves a mottled appearance. The chlorotic regions became necrotic in the course of two or three days and later the affected leaves died and usually fell from the plants. The condition extended gradually towards the apical regions of the plants, eventually causing death. A definite stunting of the roots accompanied the above symptoms.

In the case of the tomato, as in that of the bean, iron deficiency symptoms first appeared on the terminal leaves of the plants. In the tomato the early stages of the disorder, however, showed a variation from the condition observed in the bean, in that the yellowing, instead of appearing uniformly throughout the leaf tissues, started first in patches in the regions between the veins. Later development resulted in a spreading of the chlorosis, so that in a relatively short time the affected leaves became uniformly yellow in color. As in the bean, the chlorosis gradually spread downwards to the older leaves. Iron deficiency in tomato plants caused a marked stunting of both shoot and root.

Manganese deficiency effects in the tomato first became visible in the second and third leaves down from the apex. The symptoms were a very characteristic mottling of the affected leaves due to the development of chlorotic patches between the veins. As in the bean, the damage spread rapidly into the terminal leaves. Manganese deficiency retarded growth in the tomato, but the stunting was not so severe as that caused by iron deficiency.

The Absorption of Iron and Manganese from Complete Nutrient Solutions by Dwarf Bean

A number of experiments were carried out with dwarf bean plants grown in solutions which gave a wide range of variations in iron and manganese supply and of iron: manganese ratios. The results obtained from these experiments are summarized in Table IV.

An examination of these results shows that the production of iron and manganese deficiency symptoms can be correlated with two factors which are associated with the iron and manganese in the culture solutions. In the first place the actual concentrations of the two metals in the solutions are effective to the extent that if these concentrations are sufficiently low in the case of either, or both of the metals, corresponding deficiency symptoms will appear in the experimental plants. This fact is indicated by cultures 1, 5, 10, 11, and 20, as shown in Table IV. The second factor is the ratio of the concentration of iron to that of manganese in the culture solution. It appears that, provided the necessary minimum amount of each of the metals is present,

TABLE IV

DWARF BEANS, *Phaseolus vulgaris*, GROWN IN COMPLETE NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF IRON AND MANGANESE

Culture No.	Culture solutions			Symptoms*	Iron and manganese content of leaves, mgm. per gm. dry material					
	Fe, p.p.m.	Mn, p.p.m.	Fe / Mn		Fe, soluble	Fe, total	Mn, soluble	Mn, total	Sol. Fe / Sol. Mn	Total Fe / Total Mn
1	0.00	0.00	—	−Fe −Mn	0.023	0.023	0.009	0.009	—	—
2	0.00	5.00	—	−Fe	0.015	0.015	0.071	0.332	0.21	0.05
3	1.00	10.00	0.1	−Fe	0.026	0.109	0.113	0.374	0.23	0.28
4	10.00	100.00	0.1	−Fe	0.035	0.150	0.484	0.611	0.73	0.25
5	0.05	0.10	0.5	−Fe −Mn	0.014	0.014	0.016	0.016	0.88	0.88
6	5.00	10.00	0.5	−Fe	0.063	0.101	0.101	0.340	0.62	0.30
7	50.00	100.00	0.5	−Fe	0.107	0.203	0.149	0.456	0.72	0.45
8	10.00	10.00	1.0	−Fe	0.061	0.202	0.035	0.241	1.70	0.84
9	6.00	4.00	1.5	N	0.068	0.321	0.039	0.259	1.75	1.25
10	0.002	0.001	2.0	−Fe −Mn	0.044	0.044	0.000	0.000	—	—
11	0.05	0.025	2.0	−Fe −Mn	0.026	0.136	0.000	0.000	—	—
12	10.00	5.00	2.0	N	0.068	0.283	0.042	0.224	1.60	1.28
13	100.00	50.00	2.0	N	0.123	0.551	0.139	0.299	0.89	2.00
14	0.50	0.20	2.5	N	0.076	0.261	0.020	0.165	3.80	1.58
15	6.00	2.00	3.0	N	0.154	0.350	0.026	0.197	5.95	1.78
16	10.00	2.50	4.0	−Mn	0.113	0.287	0.030	0.118	3.80	2.40
17	20.00	5.00	4.0	−Mn	0.183	0.408	0.029	0.129	6.30	3.13
18	12.00	2.00	6.0	−Mn	0.196	0.393	0.020	0.113	9.80	3.50
19	10.00	1.25	8.0	−Mn	0.102	0.396	0.018	0.102	5.70	3.90
20	0.05	0.005	10.0	−Fe −Mn	0.017	0.220	0.000	0.000	—	—
21	10.00	0.50	20.0	−Mn	0.209	0.622	0.018	0.028	11.60	22.00
22	100.00	1.00	100.0	−Mn	0.226	0.796	0.020	0.037	11.30	21.50

* N = *normal*, −Fe = *iron deficiency*, −Mn = *manganese deficiency*.

the iron: manganese ratio in the culture solutions must lie between approximately 1.5 and 3.0 if healthy plants are to result. Iron: manganese ratios below this range cause iron deficiency symptoms, and above this range cause manganese deficiency symptoms, in spite of the fact that the actual concentrations of the metals may be relatively high. These results are in accordance with those obtained by Somers and Shive (8) for soybean. Somers and Shive, however, found that the optimum range in the iron to manganese ratio was also shown in the soluble iron and manganese contents of the leaves of their experimental plants. The analytical data of acetone extracts of leaf material obtained by the authors of the present paper in the case of the dwarf bean did not show this optimum range, as will be evident if the figures given in Table IV are examined. It will be seen, however, that a closer approach to an optimum ratio range was obtained from the estimations of total iron and manganese in the leaves. This optimum ratio range was narrower than that of the culture solutions and extended only from approximately 1.25 to 2.00.

An interesting relationship will be noted between the iron and manganese content of the leaves of the experimental plants and their concentrations in the culture solutions. Increase in the concentration of either one of these metals in the solution appeared to depress the accumulation of the other in the leaves. This is clearly shown by the selected figures brought together in Table V.

TABLE V

DATA SHOWING THE MUTUAL ANTAGONISM BETWEEN THE ABSORPTION OF IRON AND OF MANGANESE BY DWARF BEANS GROWN IN COMPLETE NUTRIENT SOLUTIONS

Culture No.	Culture solutions		Total Fe content of leaves, mgm. per gm. dry material	Total Mn content of leaves, mgm. per gm. dry material
	Fe, p.p.m.	Mn, p.p.m.		
4	10.0	100.00	0.150	0.611
8	10.0	10.00	0.202	0.241
12	10.0	5.00	0.283	0.224
16	10.0	2.50	0.287	0.118
19	10.0	1.25	0.396	0.102
21	10.0	0.50	0.622	0.028
3	1.00	10.00	0.109	0.374
6	5.00	10.00	0.101	0.340
8	10.00	10.00	0.202	0.241
1	0.00	5.00	0.015	0.332
12	10.00	5.00	0.283	0.224
17	20.00	5.00	0.408	0.129
15	6.00	2.00	0.350	0.197
18	12.00	2.00	0.393	0.113

It was found in the course of these experiments that the average times required for deficiency symptoms to appear were 6.3 days for iron deficiency and 10.9 days for manganese deficiency.

TABLE VI

TOMATOES, *Lycopersicum esculentum*, GROWN IN COMPLETE NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF IRON AND MANGANESE

Culture No.	Culture solutions			Symptoms*	Iron and manganese content of leaves, mgm. per gm. dry material					
	Fe, p.p.m.	Mn, p.p.m.	$\frac{Fe}{Mn}$		Fe, soluble	Fe, total	Mn, soluble	Mn, total	$\frac{Sol.\ Fe}{Sol.\ Mn}$	$\frac{Total\ Fe}{Total\ Mn}$
1	0.0	5.0	—	−Fe	0.000	0.000	0.204	0.447	—	—
2	5.0	50.0	0.1	−Fe	0.019	0.092	0.224	0.900	0.085	0.10
3	5.0	25.0	0.2	−Fe	0.031	0.136	0.176	0.787	0.18	0.17
4	5.0	10.0	0.5	N	0.092	0.397	0.164	0.636	0.56	0.63
5	5.0	5.0	1.0	N	0.110	0.474	0.150	0.501	0.96	0.95
6	10.0	5.0	2.0	N	0.149	0.574	0.098	0.387	1.49	1.48
7	25.0	5.0	5.0	N	0.158	0.698	0.032	0.129	4.95	5.40
8	50.0	5.0	10.0	−Mn	0.178	0.795	0.021	0.092	8.5	8.64
9	5.0	0.0	—	−Mn	0.077	0.340	0.000	0.000	—	—

* N = *normal*, −Fe = *iron deficiency*, −Mn = *manganese deficiency*.

The Absorption of Iron and Manganese from Complete Nutrient Solutions by Tomato

A group of water culture experiments similar to those described for the dwarf bean were carried out with tomato plants. In these a somewhat narrower range of iron and manganese supply was provided than that used in the bean cultures. The results of these experiments are summarized in Table VI.

Examination of the figures given in this table again shows that the iron: manganese concentration ratio in the culture solutions is an important factor in determining the presence or absence of deficiency symptoms. In the tomato the range of tolerance in this ratio was somewhat wider than that obtained with the bean, extending approximately from 0.5 to 5.0. It will be noted, however, that unlike what was found in the bean experiments, there was with tomatoes a fair correspondence between the ratios of soluble iron to soluble manganese, as well as of total iron to total manganese, in the leaves of the experimental plants and the ratio of these metals in the culture solutions.

It will again be seen that the mutually depressant effect of either one of the metals, iron or manganese, on the absorption of the other was clearly in evidence in the tomato experiments as it was in the bean experiments.

It was again noted that iron deficiency symptoms appeared more rapidly than manganese deficiency symptoms. Iron deficiency developed on an average in 3.5 days, while signs of manganese deficiency required an average of 20.8 days in which to appear.

The Absorption of Iron and Manganese from Solutions of Ferrous Sulphate ($FeSO_4$) and Manganous Sulphate ($MnSO_4$) by Onion Plants

Onion bulbs were grown for one month supported at the tops of beakers containing the complete culture solution (see Tables I and II, p. 563), so that their basal portions were immersed in the solution. Humate iron and manganese had previously been added to the culture solution to give a concentration of these two metals of 0.50 p.p.m. and 0.25 p.p.m. respectively. By the end of the month the bulbs had developed numerous roots of approximately two inches in length.

Four of these bulbs were then removed from the beakers of culture solution and, after their roots had been rinsed in distilled water, were given six consecutive 24 hr. periods in solutions of ferrous sulphate of concentrations which respectively provided iron contents of 20, 10, and 5 p.p.m. The roots of the bulbs were rinsed in distilled water between the changes from each of these solutions to the next. Each of the four bulbs was thus grown for 24 hr. in a solution containing 20 p.p.m. of iron, for 24 hr. in one containing 10 p.p.m. of iron, and finally for 24 hr. in one containing 5 p.p.m. The sequence was then repeated. The solutions were prepared by dissolving B.D.H. Anala R ferrous sulphate ($FeSO_4 \cdot 7H_2O$) in distilled water.

At the end of each 24 hr. run the solutions were analyzed spectrophotometrically for iron concentration.

A parallel set of experiments was carried out in an identical way with the exception that manganese (as $MnSO_4 \cdot 4H_2O$) was used instead of iron.

The averaged results of the whole series of experiments are given in Table VII. It will be noted from the figures given in this Table that, within the range of concentrations used, variations in the amounts of iron or manganese in the culture solutions did not affect the quantities of these metals absorbed by the plants.

TABLE VII

THE ABSORPTION OF IRON AND MANGANESE BY ONIONS GROWN IN SINGLE SALT SOLUTIONS OF $FeSO_4$ AND $MnSO_4$

Concentration of iron in solution, p.p.m.	Concentration of manganese in solution, p.p.m.	Average amount of iron absorbed per plant in 24 hr., mgm.	Average amount of manganese absorbed per plant in 24 hr., mgm.
20.0	—	0.73	—
10.0	—	0.72	—
5.0	—	0.73	—
—	20.0	—	0.82
—	10.0	—	0.88
—	5.0	—	0.87

A further experiment was carried out for the purpose of testing the effect of the presence of iron on the absorption of manganese and vice versa when both metals were present in the culture solutions. In this case solutions of both iron and manganese sulphates were prepared to give a range of known initial concentrations of both iron and manganese. Two sets of onion bulbs were kept with their roots in these solutions for 24 hr. periods under exactly the same conditions as described for the single salt experiments. The average amounts of iron and manganese absorbed by the bulbs from the solutions during the 24 hr. periods are given in Table VIII.

TABLE VIII

THE ABSORPTION OF IRON AND MANGANESE BY ONIONS GROWN IN SOLUTIONS CONTAINING BOTH $FeSO_4$ AND $MnSO_4$

Initial concentrations of Fe and Mn in solutions, p.p.m.		Fe and Mn absorbed per bulb in 24 hr., p.p.m.		
Fe	Mn	Fe	Mn	Fe + Mn
5	25	0.13	0.60	0.73
5	10	0.23	0.51	0.74
5	5	0.29	0.38	0.67
10	5	0.42	0.33	0.75
25	5	0.54	0.18	0.73

It will be noted from the data given in Table VIII that the apparent antagonism between the absorption of iron and of manganese that was observed in the previous experiments with bean and tomato plants grown in

complete nutrient solutions is again shown in the case of onions growing in solutions containing only iron and manganese salts.

The figures indicating the combined amounts of iron and manganese absorbed show that this amount is relatively constant irrespective of the initial concentration ratios of the two metals in the solutions.

Discussion

The experiments here described uphold the view that both the absolute amounts of iron and manganese and the concentration ratios of these two metals in the culture solutions are related to the production of deficiency symptoms.

The analytical data for soluble iron and soluble manganese in the leaves of the experimental plants do not furnish any very definite support for the oxidation–reduction hypothesis put forward by Somers and Shive (8).

The definite relationship that is exhibited between the absorption of iron and of manganese appears to the authors to be of considerable interest. The low level of the concentrations of these metals in most of the culture solutions would seem to indicate that we are not dealing with a case of antagonism as it is usually understood. Further, there is revealed by the onion experiments the extraordinary fact that the total amount of iron plus manganese absorbed remained virtually unchanged irrespective of the values of the concentration ratios of the two metals in the culture solutions. The meaning of this is obscure, though it does suggest that there should be some degree of interchangeability between the functions of the two metals. The fact that iron and manganese are not interchangeable, however, is clearly indicated by the experiments in which deficiency symptoms are produced.

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THE EFFECT OF INFECTION BY *HELMINTHOSPORIUM SATIVUM* ON THE AMINO ACID CONTENT OF WHEAT ROOTS[1]

By S. B. Hrushovetz[2]

Abstract

The amino acids present in roots of healthy and diseased (*Helminthosporium sativum* P. K. & B.) wheat seedlings were determined by paper chromatography. Treatment of dried roots with 80% ethanol followed by acid hydrolysis effected maximum extraction of the amino acids. Eleven free and 13 combined amino acids were identified on the chromatograms, and four unidentified fractions were indicated. Total nitrogen of the diseased roots was higher on a dry weight basis than that of healthy roots. The aggregate content of both free and combined amino acids was slightly higher in the diseased roots than in the healthy ones. However, the concentrations of free alanine, serine, and asparagine in the diseased roots were twice those in the healthy ones.

Introduction

It is only within recent years that paper chromatography has been applied to the analysis of organic compounds occurring in plants (5). Andreae and Thompson (2) found a higher concentration of free tyrosine and tryptophane in healthy potato tubers than in tubers infected with leaf-roll virus. In root nodules of the alfalfa plant, Hunt (7) found free tryptophane and certain basic amino acids that were absent from the roots proper. Allsopp (1) found a higher concentration of free amino acids in the root apices of certain ferns than in the general root tissue. Steinberg (9) has suggested that the expression of disease symptoms in plants may be associated with localization of abnormal concentrations of amino acids. The present investigation concerns the identification and estimation of the amino acids as found in healthy and diseased roots of wheat seedlings.

Methods

Surface-disinfected grains of Thatcher wheat were planted in an unsterilized mixture of black loam and sand in 6-in. pots in the greenhouse. Diseased roots were secured from this soil artificially infested with *Helminthosporium sativum*, and healthy ones from plants grown in noninfested soil. The plants were harvested after two weeks' growth, the roots were washed, and the first two inches below the crown removed. Only portions of the diseased roots showing lesions that were typical of those caused by *H. sativum* were used for analysis of free and combined amino acids.

For the detection of amino acids in the wheat roots the technique of paper chromatography as introduced by Consden *et al.* (4) was employed.

[1] *Manuscript received in original form March 17, 1953, and, as revised, January 18, 1954. Contribution No. 1393 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada. This paper is a portion of a thesis submitted to the Department of Biochemistry, University of Alberta, in partial fulfillment of the requirements for the degree of Master of Science.*

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Free amino acids were extracted by a modification of the method of Dent *et al.* (5). Composite samples of fresh roots were dried overnight in an oven at 90° C. and then ground in a mortar. Two milliliters of 80% ethanol was then added to 10 mgm. of the dried ground material and the mixture was placed in the refrigerator for 24 hr. and shaken at intervals. The extraction with ethanol diminishes interference by substances such as inorganic ions and certain proteins. Finally the supernatant fluid was removed with a pipette, and 1.0 ml. was applied as a small spot near the corner of a sheet of Whatman No. 1 filter paper, size $18\frac{1}{2} \times 22$ in. The two-dimensional chromatogram was developed with *n*-butanol – glacial acetic acid – water (4 : 1 : 5 by volume) as the first solvent and 80% phenol–water as the second solvent. The position of the amino acids on the paper was revealed by spraying with a 0.2% solution of ninhydrin in anhydrous butanol.

For determination of combined amino acids in the roots, 10 mgm. of dried material was extracted with 2 ml. of 80% ethanol as before. The residue was then treated with 2 ml. of 6 *N* hydrochloric acid and hydrolyzed in the autoclave overnight at 12 lb. pressure. The hydrochloric acid was evaporated in the oven at 90° F. and the black residue was extracted with 2 ml. of 80% ethanol. The chromatograms were spotted with 0.60 ml. of the extract in a current of warm air to prevent concentration of the extract and developed as above.

The identities of the amino acids, which appear as red or purple areas on the chromatograms, were established by preparing chromatograms with known amino acids. Furthermore, a set of chromatograms was prepared by using varying quantities of the pure amino acids. The concentration of the amino acids in these standard chromatograms varied from 5 to 60 μgm. It was found that the surface area and color intensity of the spots, after treatment with ninhydrin, varied with the concentration of the amino acid. Four chromatograms were found to cover this range satisfactorily. However, for concentrations above 60 μgm. the spots of most amino acids became diffuse and irregular in outline, especially when the butanol – acetic acid – water solvent was used. The volume of extract spotted was adjusted so that no amino acid appeared to have a value greater than 60 μgm. on the chromatograms. This method of visual comparison of spots with standards developed under identical conditions was used for the quantitative analysis of amino acids in silk hydrolyzates by Polson *et al.* (8).

Thompson *et al.* (10) discuss the merits of various quantitative procedures. They found that acetone extraction of the colored ninhydrin – amino acid complex was not a reliable procedure. The author has tried Woiwood's copper phosphate method (11) for two-dimensional chromatograms but found it unsatisfactory. Awapara (3), who completed the ninhydrin reaction in a test tube, got a coefficient of variation of 5% for 20 separate determinations. In his studies Awapara was concerned with only four amino acids and he did not analyze quantitatively those found in low concentrations. The spectrophotometric methods as outlined by Fitzpatrick (6) or Thompson *et al.* (10)

could not be utilized in this study because with many amino acids the concentration was far too low. These authors employed concentrations of 40 to 50 μgm. In chromatograms of extracts of wheat roots studied, leucine, valine, and other amino acids were present in 10 to 15 μgm. quantities. It is for these reasons that the subjective method of Polson *et al.* (8) was used. Preliminary experiments showed errors of 5–8% with identical chromatograms. Verification of this variation was obtained when total amino acids, i.e. a chromatogram containing both the free and combined amino acids, was compared with the aggregate amino acids, i.e. a chromatogram containing the free amino acids plus a chromatogram containing the combined amino acids. Consequently, only differences greater than 0.5 mgm. per gm. dry tissue were considered as significant. Approximately 100 chromatograms were developed from several different samples and inspection showed that the diseased samples invariably gave chromatograms with more intense spots. The semiquantitative data shown in Table I are based on four chromatograms prepared under standard conditions.

Results

The chromatograms of the free and combined amino acids in healthy and diseased wheat roots are reproduced in Plate I. The amino acids that correspond to the numbers used in the chromatograms are listed in Table II, and the concentrations of the various amino acids in relation to the surface area and intensity of the spots (8) are shown in Table I and in Plate I.

In Plate I spot *G* is the alternate position of glutamic acid. When present in higher concentration this amino acid appears in two separate spots. The basic amino acids arginine, lysine, and histidine appear in separate distinct spots when the pure chemicals are used, but with extracts the spots are indistinct and merge together. Consequently the positions 1 and 19 were tentatively identified and their combined concentrations were expressed in Table I by the use of the sign '+'. Various unknown ninhydrin-positive spots also occurred. Spots *H* and *Y* are basic amino acid derivatives. Spots *B* and *C* are probably members of some homologous series. Their position suggests that they may be 'peptide' derivatives that have formed as a result of prolonged acid hydrolysis.

In Table I it is seen that the aggregate content of both free and combined amino acids, expressed as milligrams per gram of dried tissue, is slightly higher in the diseased roots than in the healthy roots. The concentrations of alanine, serine, and asparagine were, however, definitely greater. Also, Kjeldahl determinations showed 7.96% and 6.0% of total nitrogen in dried diseased and healthy root tissue, respectively. Carbon content of the diseased roots was also slightly greater than that of the healthy ones, the respective analyses showing 37.7% and 37.0%.

Not all the amino acids occurred in higher concentration in the diseased root tissue. Amino butyric acid and phenylalanine were in greater concentration in the healthy tissue. The basic amino acids were also in greater amounts

TABLE I

CONCENTRATION OF FREE AND COMBINED AMINO ACIDS IN HEALTHY AND DISEASED WHEAT ROOTS, EXPRESSED AS MILLIGRAMS PER GRAM OF DRIED TISSUE

Amino acid	Healthy		Diseased	
	Free	Combined	Free	Combined
Alanine	2.0	6.0	4.0	13.0
Arginine, Histidine, Lysine	+*	+++	++	++
Asparagine	6.0	—	12.0	—
Aspartic acid	3.0	5.0	5.0	6.0
Glutamic acid	4.0	8.5	4.0	8.5
G**	—	3.5	—	5.0
Glycine	—	4.0	—	5.0
Glutamine	8.0	6.0	8.0	6.0
Leucine	1.0	6.0	1.5	8.5
Phenylalanine	—	6.0	—	3.5
Proline	—	5.0	—	6.0
Serine	1.5	5.0	4.0	6.0
Threonine	2.0	3.5	2.5	5.0
Tyrosine	—	3.5	—	5.0
Valine	1.0	2.0	1.5	1.5
Amino butyric acid	2.0	—	1.5	—

* *Has quantitative significance. The three amino acids occurred in one spot and it was not possible to estimate them separately.*

** *Alternate position of glutamic acid.*

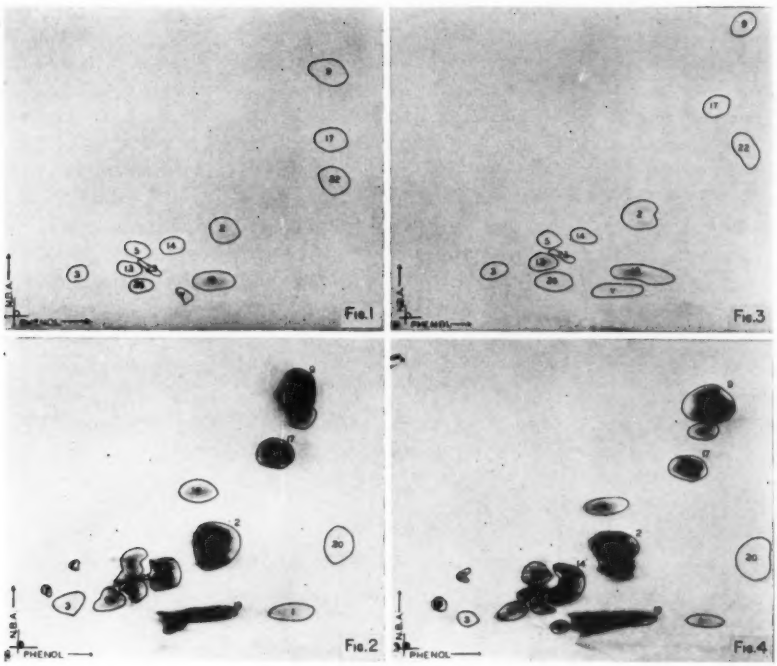
TABLE II

IDENTIFICATION KEY OF AMINO ACIDS SHOWN ON CHROMATOGRAMS IN PLATE I

No.	Amino acid	No.	Amino acid
1	Arginine	14	Threonine
2	Alanine	16	Tyrosine
3	Aspartic acid	17	Valine
5	Glutamic acid	19	Lysine
6	Glycine	20	Proline
9	Leucine	22	Amino butyric acid
12	Phenylalanine	23	Glutamine
13	Serine	26	Asparagine

in the combined form in the healthy tissue. Phenylalanine and proline could not be detected in the free state in either the healthy or diseased roots. Preliminary experiments showed that the amino acids phenylalanine and proline were very abundant in mycelial extracts of *H. sativum* in the free state. As these amino acids could not be detected in the extract of diseased roots, it would appear that the mycelium of *H. sativum* present in the diseased root

PLATE I



FIGS. 1 to 4. Comparative chromatograms of extracts from healthy (*left*) and diseased (*Helminthosporium sativum*) (*right*) Thatcher wheat roots. Solvents were *n*-butanol – glacial acetic acid – water (4 : 1 : 5), and 80% aqueous phenol solution.

Note deeper color of Figs. 2 and 4, *combined amino acids*, obtained from 0.60 ml. of extract using 80% ethanol followed by acid hydrolysis, than of Figs. 1 and 3, *free amino acids*, where 1.0 ml. of unhydrolyzed extract was employed.

samples was of insufficient mass to show a detectable quantity of phenylalanine or proline. Likewise it is unlikely that any significant amounts of other amino acids could have been contributed by the fungus mycelium.

Discussion

Serine, alanine, and asparagine were found in higher concentrations in the diseased than in the healthy root tissue of Thatcher wheat. The remainder of the detectable amino acids either showed no difference or even appeared to be slightly higher in concentration in the healthy tissue. With the method of quantitative analysis employed, it is unknown whether the findings mentioned in the last statement are significant. Steinberg (9), in discussing the expression of frenching symptoms in tobacco plants, has suggested that disease symptoms may be caused by the localization of abnormal concentrations of the amino acids proline and hydroxyproline. It is apparent from the present study that the higher concentrations of serine, alanine, and asparagine in the diseased root tissue are associated with the root rot symptoms. The presence of the fungus mycelium on or inside the root tissue must in some way upset the protein metabolism in the wheat seedling so that there is an increase both in total and amino nitrogen, possibly by increasing absorption of nitrates from the soil or by preventing normal translocation to other parts of the plant.

Acknowledgments

The author is grateful for the valuable assistance of his adviser, Dr. H. B. Collier, head of the Department of Biochemistry, University of Alberta, and also for that of Dr. L. E. Tyner of this laboratory.

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THE OCCURRENCE OF *FUSARIUM* SPECIES IN CANADA

III. TAXONOMY OF *FUSARIUM* SPECIES IN THE SEED OF VEGETABLE, FORAGE, AND MISCELLANEOUS CROPS[1]

By W. L. Gordon[2]

Abstract

A taxonomic study was made of isolates of *Fusarium* obtained from a microbiological examination of seed from a total of 44 species and botanical varieties of cultivated plants. These plants may be classed as vegetable, forage, and miscellaneous crops. Most of the seed samples originated from stocks produced in Canada, but some were seed from other countries. A total of 16 species, varieties, and forms of *Fusarium*, classified in eight sections of the genus, were encountered among the isolates that were obtained from seed; they are *F. poae* (Pk.) Wr., *F. sporotrichioides* Sherb., *F. arthrosporioides* Sherb., *F. avenaceum* (Fr.) Sacc., *F. semitectum* Berk. & Rav., *F. acuminatum* Ell. & Ev., *F. compactum* (Wr.) Gordon, *F. equiseti* (Cda.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. sambucinum* Fuckel, *F. sambucinum* var. *coeruleum* Wr., *F. moniliforme* Sheld. em. S. & H., *F. oxysporum* Schlecht. em. S. & H., *F. oxysporum* f. *lini* (Bolley) S. & H., *F. solani* (Mart.) App. & Wr. em. S. & H. From the seed of a total of 44 species and botanical varieties of host plants, *F. equiseti* was identified among isolates from 34; *F. poae* and *F. oxysporum* each from 18; *F. acuminatum* from 17; *F. avenaceum* from 16; *F. culmorum* from 10; *F. sambucinum* and *F. moniliforme* each from five; *F. graminearum* and *F. sambucinum* var. *coeruleum* each from four; *F. solani* from three; *F. sporotrichioides* from two; *F. arthrosporioides*, *F. semitectum*, *F. compactum*, and *F. oxysporum* f. *lini* each from one. The origin of the seed samples of each host is given.

Introduction

The increased emphasis that was placed on quality in agricultural seed during the war years, 1939–1945, led to the expansion of microbiological seed-testing in Canada. As a result of the employment of this type of seed-testing, it was found that a wide variety of fungi were harbored by the seed of various agricultural crops even after the surface of the seed was disinfected. Among the fungi obtained there was an appreciable number of isolates of *Fusarium*. A taxonomic study of the *Fusarium* isolates from seed of wheat, oats, and barley (1) showed that they consisted of a total of 16 species, varieties, and forms, among which were the more important species previously known to be capable of causing damage to cereals. As information with respect to the identity of the *Fusarium* species that might be harbored by locally grown or imported seed of noncereal crops was quite meager, a further taxonomic study was made of isolates of *Fusarium* obtained from seed of certain vegetable, forage, and miscellaneous crops.

Materials and Methods

The isolates of *Fusarium* on which the present taxonomic study was made were obtained from two sources. Most of the isolates were obtained from Dr. J. W. Groves and Dr. A. J. Skolko, Plant Pathology Laboratory, Ottawa,

[1] *Manuscript received April 28, 1954.*
Contribution No. 1387 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.
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Ont., who had encountered them among fungi which they had obtained during their extensive microbiological examination of samples of seed of various cultivated crops. Details of the scope of their investigation on seed-borne fungi and their method of surface disinfection of the seed have already been published (2). Although the majority of the seed samples from which they obtained isolates of *Fusarium* were of Canadian origin, some samples originated in certain other countries, namely, United States, England, Scotland, Denmark, Holland, and U.S.S.R. In addition to the sources just mentioned, other isolates were obtained at the Plant Pathology Laboratory, Winnipeg, from a microbiological examination of occasional samples of seed of miscellaneous crops. These samples were subjected to surface disinfection, previous to plating, by immersion in a mercuric chloride/alcohol solution for three to five minutes, followed by a rinse in sterile water.

The various isolates of *Fusarium* included in this study were obtained from the seed of a total of 44 species and botanical varieties of cultivated plants representing vegetable, forage, and miscellaneous crops.

The culture technique and the method of determining growth rates and of making measurements and drawings employed during this taxonomic study were essentially the same as those used in a previous taxonomic study of *Fusarium* isolates obtained from cereal seed (1). Monoconidial cultures on potato sucrose agar, slanted in 1 in. × 8 in. test tubes were used for making photographs. The age of the cultures when photographed was 10 days.

Classification of the *Fusarium* Isolates

The naming and classifying of the *Fusarium* isolates obtained from non-cereal crops was carried out in accordance with the method adopted for those obtained from cereal crops (1). By this means a total of 16 species, varieties, and forms of *Fusarium* were identified, representing eight sections of the genus, namely, Sporotrichiella, Roseum, Arthrosporiella, Gibbosum, Discolor, Liseola, Elegans, and Martiella.

The synonymy of the species, varieties, and forms identified in this study, when not given, can be found in previous publications (1, 5, 6, 7, 8, 9).

Section Sporotrichiella Wr.

Wollenweber, apud Lewis in Maine Agr. Expt. Sta. Bull. 219 : 256. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 45–47. 1935.
Wollenweber, Fusarium-Monographie II, pp. 125–127. 1944–45.

Two species of *Fusarium*, namely, *F. poae* (Pk.) Wr. and *F. sporotrichioides* Sherb., classified in this section of the genus, were encountered among the isolates from the seed of several species of host plants. *F. poae* was isolated from the seed of a total of 18 species of plants whereas *F. sporotrichioides* was isolated only from the seed of two species. *F. poae* is encountered in nature more frequently than *F. sporotrichioides* and was previously found to be the most commonly isolated species from the seed of wheat, barley, and oats in Canada (1). As this species is not generally considered to be an aggressive

parasite, its frequent occurrence in association with seeds is probably more dependent on environmental factors favorable to seed infection prevailing during the period of seed development and maturation than on its virulence as a parasite.

FUSARIUM POAE (Pk.) Wr.

Wollenweber and Reinking, Die Fusarien, p. 47. 1935.
Wollenweber, Fusarium-Monographie II, pp. 127–128. 1944–45.
Gordon, Can. J. Botany, 30 : 219. 1952.

This species was isolated from the seed of *Apium graveolens* L. var. *dulce* DC. (celery), *Beta vulgaris* L. (beet), *Bromus inermis* Leyss. (brome grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Glycine max* Merr. (soybean), *Linum usitatissimum* L. (flax), *Nicotiana tabacum* L. (tobacco), *Panicum miliaceum* L. (millet), *Pastinaca sativa* L. (parsnip), *Phaseolus vulgaris* L. (bean), *Pisum sativum* L. (pea), *Raphanus sativus* L. (radish), *Secale cereale* L. (rye), *Spinacia oleracea* L. (spinach), *Trifolium hybridum* L. (alsike), *Trifolium pratense* L. (red clover), *Vicia faba* L. (broad bean), and *Zea mays* L. (corn).

FUSARIUM SPOROTRICHIOIDES Sherb.

Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6 : 183–186. 1915.
Wollenweber and Reinking, Die Fusarien, pp. 48–49. 1935.
Wollenweber, Fusarium-Monographie II, pp. 129–130. 1944–45.
Gordon, Can. J. Botany, 30 : 219–220. 1952.

This species was isolated only from the seed of *Cucurbita pepo* L. (pumpkin) and *Pisum sativum* L. (pea).

Section Roseum Wr.

Wollenweber, Phytopath. 3 : 32. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 49–53. 1935.
Wollenweber, Fusarium-Monographie II, pp. 130–131. 1944–45.
Snyder and Hansen, Am. J. Botany, 32 : 663. 1945.

Two species of *Fusarium*, namely, *F. arthrosporioides* Sherb. and *F. avenaceum* (Fr.) Sacc., belonging in this section of the genus, were encountered among the isolates from the seed of several different crops. *F. avenaceum*, however, was much more commonly encountered than *F. arthrosporioides*; the former species was isolated from the seed of 16 species of plants, the latter only from the seed of a single host. *F. arthrosporioides* would appear to be seldom seed-borne by cultivated plants as it also occurred rarely in cereal seed (1).

FUSARIUM ARTHROSPORIOIDES Sherb.

Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6 : 175–179. 1915.
Wollenweber and Reinking, Die Fusarien, pp. 56–57. 1935.
Wollenweber, Fusarium-Monographie II, pp. 173–174. 1944–45.
Gordon, Can. J. Botany, 30 : 221–222. 1952.

This species was isolated only from the seed of *Lolium perenne* L. (rye grass).

FUSARIUM AVENACEUM (Fr.) Sacc.

Saccardo, Syll. Fungorum, 4 : 713. 1886.
Wollenweber and Reinking, Die Fusarien, pp. 53–55. 1935.
Wollenweber, Fusarium-Monographie II, pp. 132–135. 1944–45.
Gordon, Can. J. Botany, 30 : 221. 1952.

F. avenaceum was present among isolates obtained from seed of the following crops: *Allium cepa* L. (onion), *Asparagus officinalis* L. var. *altilis* L. (asparagus), *Beta vulgaris* L. (mangel), *Brassica napobrassica* Mill. (swede), *Cucurbita maxima* Duchesne (squash), *Daucus carota* L. var. *sativa* DC. (carrot), *Linum usitatissimum* L. (flax), *Lolium perenne* L. (rye grass), *Medicago sativa* L. (alfalfa), *Pisum sativum* L. (pea), *Raphanus sativus* L. (radish), *Secale cereale* L. (rye), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod. (Russian dandelion), *Trifolium hybridum* L. (alsike), and *Trifolium pratense* L. (red clover).

Section Arthrosporiella Sherb.

Sherbakoff, N.Y. Cornell Agr. Expt. Sta. Memoir, 6 : 161. 1915.
Wollenweber, Fusarium-Monographie, pp. 322–324. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 57–58. 1935.
Snyder and Hansen, Am. J. Botany, 32 : 663. 1945.

Only a single representative of this section of the genus, namely, *F. semitectum* Berk. & Rav., was encountered during this study.

FUSARIUM SEMITECTUM Berk. & Rav.

Berkeley, Notices of North American fungi. Grevillea, 3 : 98. 1875.
Wollenweber, Fusarium-Monographie, pp. 324–325. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 58–59. 1935.
Gordon, Can. J. Botany, 30 : 222–223. 1952.

This species was isolated only from the seed of *Pisum sativum* L. (pea).

Section Gibbosum Wr.

Wollenweber, Phytopath. 3 : 31. 1913.
Wollenweber, Fusarium-Monographie, pp. 328–330. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 61–62. 1935.
Snyder and Hansen, Am. J. Botany, 32 : 663. 1945.
Gordon, Can. J. Botany, 30 : 223–224. 1952.

Three species of *Fusarium*, namely, *F. acuminatum* Ell. & Ev., *F. compactum* (Wr.) Gordon, and *F. equiseti* (Cda.) Sacc., classified in this section of the genus, were present among the isolates obtained from the seed of several different crops. *F. equiseti* was isolated from the seed of 34 of the 44 crops that yielded isolates of *Fusarium*. This species was encountered among the isolates from the seed of a larger number of crops than any of the other species of *Fusarium* obtained during this study. *F. acuminatum* also was found to be rather widely seed-borne; it was isolated from the seed of a total of 17 different crops. On the other hand, *F. compactum* was isolated from the seed of only one crop.

FUSARIUM ACUMINATUM Ell. & Ev.

Ellis and Everhart, Proc. Acad. Sci. Philadelphia, p. 441. 1895.

Wollenweber, J. Agr. Research, 2 : 269–270. 1914.

Gordon, Can. J. Botany, 30 : 226. 1952.

This species was isolated from the seed of *Agropyron cristatum* (L.) Beauv. (crested wheat grass), *Allium cepa* L. (onion), *Apium graveolens* L. var. *dulce* DC. (celery), *Beta vulgaris* L. (beet), *Bromus inermis* Leyss. (brome grass), *Carthamus tinctorius* L. (safflower), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca rubra* L. (red fescue), *Linum usitatissimum* L. (flax), *Phaseolus vulgaris* L. (bean), *Pisum sativum* L. (pea), *Secale cereale* L. (rye), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod. (Russian dandelion), *Trifolium hybridum* L. (alsike), *Vicia faba* L. (broad bean), and *Zea mays* L. (corn).

FUSARIUM COMPACTUM (Wr.) Gordon

Gordon, Can. J. Botany, 30 : 224. 1952.

Syn. *F. scirpi* Lamb. & Fautr. var. *compactum* Wr.

Wollenweber, Fusarium-Monographie, p. 333. 1931.

F. scirpi Lamb. & Fautr. var. *compactum* Wr. pr. p.

Wollenweber and Reinking, Die Fusarien, pp. 66–67. 1935.

F. roseum Lk. emend. Snyder & Hansen pr. p.

Snyder and Hansen, Am. J. Botany, 32 : 663. 1945.

The taxonomy of *F. compactum* was discussed in a recent paper (1). Certain data are now presented with respect to the cultural and morphological characteristics of the wild type of this species and of a striking cultural mutant. The greater length of the conidia of the mutant, partially due to the elongated apical cell, shows its affinity to the section Gibbosum more markedly than does the wild type from which it originated.

Conidia and Chlamydospores

Conidia of the wild type (Fig. 1).

Conidia 5(1–3–5)-septate; 1-sept. 14.8 – 21.2 × 7.4 – 10.6
3-sept. 19.1 – 25.4 × 4.2 – 9.5
5-sept. 29.7 – 53.0 × 4.7 – 6.4

Chlamydospores of the wild type intercalary, singly, in pairs, and in chains (Fig. 2, *a*, *b*, *c*).

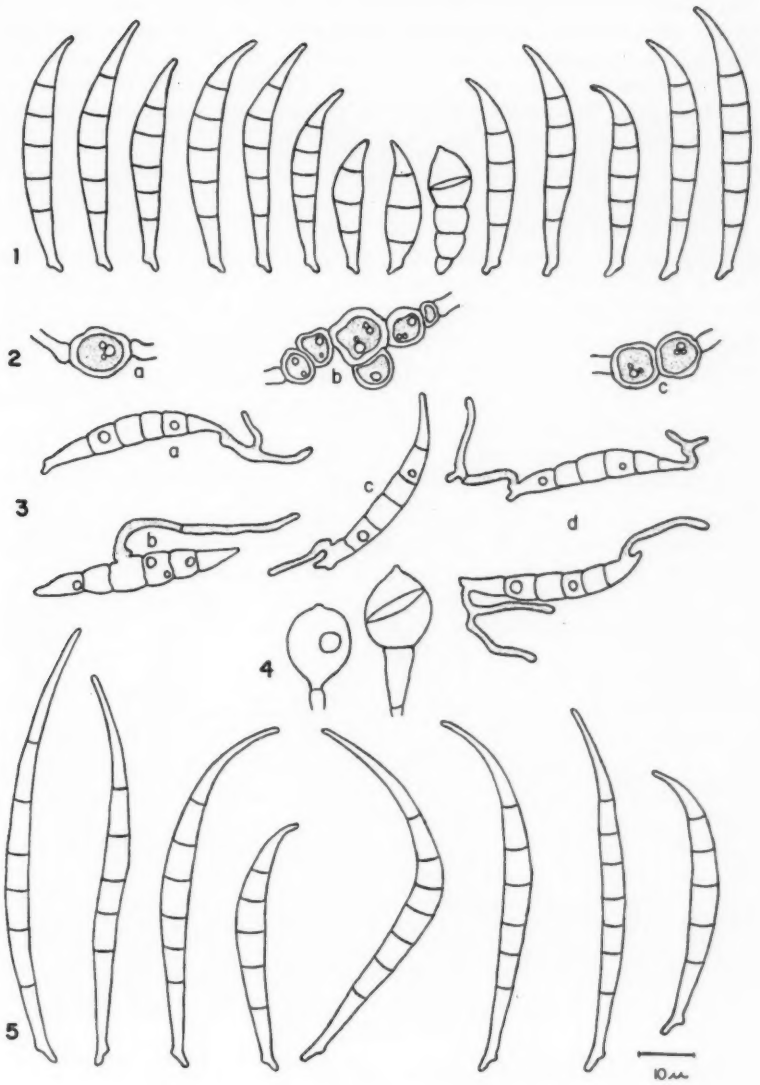
Growth rate of the wild type 4.6 cm.

Conidia of the mutant (Fig. 5).

Conidia 5(5–6–7)-septate; 5-sept. 42.4 – 63.6 × 4.9 – 6.4
6-sept. 48.8 – 70.0 × 4.5 – 6.4
7-sept. 70.0 – 84.8 × 4.3 – 6.4

Chlamydospores of the mutant as in the wild type.

Growth rate of the mutant 5.3 cm.



FIGS. 1–5. *F. compactum*. FIG. 1. Conidia of wild type. FIG. 2. Chlamydospores: *a*. a single chlamydospore; *b*. chlamydospores in a chain; *c*. a pair of chlamydospores. FIG. 3. Germination of conidia of the wild type: *a*. from the apical cell; *b*. from an intercalary cell; *c*. from the basal cell; *d*. simultaneously from basal and apical cells. FIG. 4. Swellings at end of hyphae. Fig. 5. Conidia of the cultural mutant.

Germination of conidia of the wild type takes place as shown in Fig. 3. The first germ tube may arise from the apical cell (Fig. 3*a*), from an intercalary cell (Fig. 3*b*), from the pedicellate basal cell (Fig. 3*c*), or from the apical and basal cells more or less simultaneously (Fig. 3*d*). The conidia of the mutant showed the same variation in the manner of germination as the wild type.

Terminal swellings (Fig. 4) were frequently observed at the end of hyphae in both the wild type and mutant. The cause of these swollen cells was not determined.

There is a marked difference in the appearance of cultures of the wild type (Fig. 6*a*) and those of the mutant (Fig. 6*c*) owing to the profuse development of sporodochia and pionottes by the mutant. Stroma color in both the wild type and the mutant is bright carmine (Fig. 6*b*).

F. compactum was isolated only from imported seed of *Arachis hypogaea* L. (peanut). This species has not been isolated previously in Canada.

FUSARIUM EQUISETI (Cda.) Sacc.

Saccardo, Syll. Fungorum 4, pp. 707–708. 1886.
Wollenweber and Reinking, Die Fusarien, pp. 63–64. 1935.
Gordon, Can. J. Botany, 30 : 225–226. 1952.

This species was isolated from the seed of *Allium cepa* L. (onion), *Apium graveolens* L. var. *dulce* DC. (celery), *Arachis hypogaea* L. (peanut), *Asparagus officinalis* L. var. *altilis* L. (asparagus), *Beta vulgaris* L. (beet, mangel), *Brassica napobrassica* Mill. (swede), *Brassica oleracea* L. var. *capitata* L. (cabbage), *Brassica oleracea* L. var. *botrytis* L. (cauliflower), *Bromus inermis* Leyss. (brome grass), *Cucumis melo* L. (muskmelon), *Cucurbita pepo* L. (pumpkin, vegetable marrow), *Cucurbita maxima* Duchesne (squash), *Daucus carota* L. var. *sativa* DC. (carrot), *Festuca elatior* L. (meadow fescue), *Festuca rubra* (red fescue), *Glycine max* Merr. (soybean), *Hibiscus esculentus* L. (okra), *Hyoscyamus niger* L. (henbane), *Linum usitatissimum* L. (flax), *Lolium perenne* L. (rye grass), *Medicago sativa* L. (alfalfa), *Panicum miliaceum* L. (millet), *Pastinaca sativa* L. (parsnip), *Phaseolus vulgaris* L. (bean), *Phleum pratense* L. (timothy), *Pisum sativum* L. (pea), *Raphanus sativus* L. (radish), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod. (Russian dandelion), *Tetragonia expansa* Murr. (New Zealand spinach), *Trifolium hybridum* L. (alsike), *Trifolium pratense* L. (red clover), *Vicia faba* L. (broad bean), and *Zea mays* L. (corn).

Section Discolor Wr.

Wollenweber, Phytopath. 3 : 31. 1913.
Wollenweber, Fusarium-Monographie, pp. 346–349. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 69–72. 1935.
Snyder and Hansen, Am. J. Botany, 32 : 663. 1945.

Four species and varieties of *Fusarium* belonging to this section of the genus were identified among the isolates obtained from the seed of several species of

PLATE I

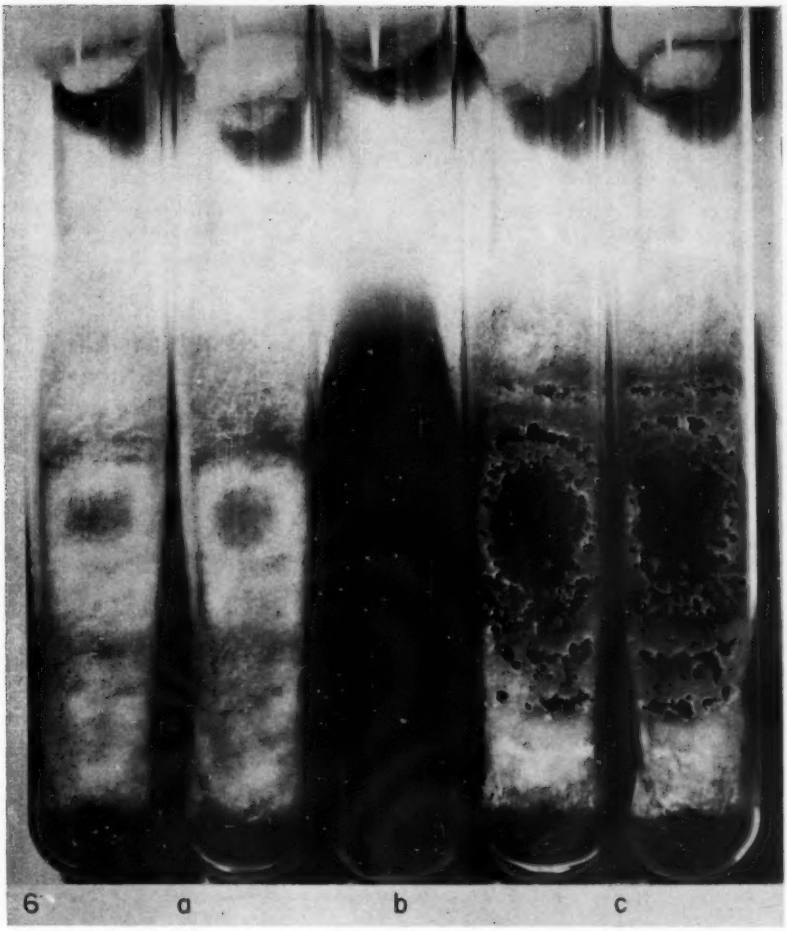


FIG. 6. Cultural characteristics of the wild type and cultural mutant of *F. compactum*: *a*. two monoconidial cultures of the wild type; *b*. intensity of color (bright carmine) in the stroma, typical of the wild type and mutant; *c*. two monoconidial cultures of the mutant.

cultivated plants. *F. culmorum* was isolated from the seed of 10 species; *F. sambucinum* from the seed of five; and *F. graminearum* and *F. sambucinum* var. *coeruleum* each from the seed of four species.

FUSARIUM CULMORUM (W. G. Sm.) Sacc.

Saccardo, Syll. Fungorum, 11 : 651–652. 1895.
Wollenweber and Reinking, Die Fusarien, pp. 79–81. 1935.
Gordon, Can. J. Botany, 30 : 227–228. 1952.

This species was identified among isolates obtained from the seed of *Agropyron trachycaulum* (Link) Malte (Western rye grass), *Beta vulgaris* L. (beet), *Bromus inermis* Leyss. (brome grass), *Daucus carota* L. var. *sativa* DC. (carrot), *Linum usitatissimum* L. (flax), *Lolium perenne* L. (rye grass), *Phaseolus coccineus* L. (scarlet runner bean), *Phaseolus vulgaris* L. (bean), *Pisum sativum* L. (pea), and *Spinacia oleracea* L. (spinach).

FUSARIUM GRAMINEARUM Schwabe

Wollenweber, Fusarium-Monographie, pp. 362–363. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 82–83. 1935.
Gordon, Can. J. Botany, 30 : 228. 1952.

This well-known pathogen of cereals was identified only from the seed of *Linum usitatissimum* L. (flax), *Pisum sativum* L. (pea), *Trifolium pratense* L. (red clover), and *Zea mays* L. (corn).

FUSARIUM SAMBUCINUM Fuckel

Wollenweber, Fusarium-Monographie, pp. 352–353. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 75–76. 1935.
Gordon, Can. J. Botany, 30 : 229–230. 1952.

This species was identified among the isolates obtained from the seed of *Brassica oleracea* L. var. *botrytis* L. (cauliflower), *Cucumis melo* L. (muskmelon), *Daucus carota* L. var. *sativa* DC. (carrot), *Hyoscyamus niger* L. (henbane), and *Taraxacum kok-saghyz* Rod. (Russian dandelion).

FUSARIUM SAMBUCINUM Fuckel var. *coeruleum* Wr.

Wollenweber, Ann. Mycol. 15 : 55. 1917.
Gordon, Can. J. Botany, 30 : 230. 1952.

This variety of *F. sambucinum* was isolated from the seed of *Agropyron cristatum* (L.) Beauv. (crested wheat grass), *Beta vulgaris* L. (mangel), *Brassica napobrassica* Mill. (swede), and *Cucurbita pepo* L. (vegetable marrow).

Section Liseola Wr., Sherb., Rg., Johann, & Bailey

Wollenweber, Sherbakoff, Reinking, Johann, & Bailey, J. Agr. Research, 30 : 841. 1925.
Wollenweber, Fusarium-Monographie, pp. 388-391. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 96–98. 1935.
Snyder and Hansen, Am. J. Botany, 32 : 664. 1945.

FUSARIUM MONILIFORME Sheld. emend. Snyder & Hansen

Sheldon, Nebraska Agr. Expt. Sta. Ann. Rept. 17 : 23–32. 1904.
Wollenweber, Fusarium-Monographie, pp. 391–393. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 98–99. 1935.
Snyder and Hansen, Am. J. Botany, 32 : 664. 1945.
Gordon, Can. J. Botany, 30 : 234–235. 1952.

This species is widely known owing to its frequent association with the seed of corn (*Zea mays* L.). It was identified among isolates from the seed of *Allium cepa* L. (onion), *Glycine max* Merr. (soybean), *Pisum sativum* L. (pea), *Trifolium hybridum* L. (alsike), and *Zea mays* L. (corn).

Section Elegans Wr.

Wollenweber, Phytopath. 3 : 28. 1913.
Wollenweber, Fusarium-Monographie, pp. 400–406. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 104–109. 1935.
Snyder and Hansen, Am. J. Botany, 27 : 64–67. 1940.
Gordon, Can. J. Botany, 30 : 236–237. 1952.

FUSARIUM OXYSPORUM Schlecht. emend. Snyder & Hansen

Wollenweber, Fusarium-Monographie, pp. 416–418. 1931.
Wollenweber and Reinking, Die Fusarien, pp. 117–118. 1935.
Snyder and Hansen, Am. J. Botany, 27 : 66. 1940.
Gordon, Can. J. Botany, 30 : 236–237. 1952.

This species is world-wide in its distribution, and is of distinct economic importance because of the ability of its specialized forms or races to cause wilting in various species of cultivated plants. During this study, *F. oxysporum* was identified among isolates from the seed of 18 species of cultivated plants. Only one species, namely, *F. equiseti* (Section Gibbosum), was isolated from the seed of a greater number of different species of host plants.

Isolates of *F. oxysporum* were obtained from the seed of *Allium cepa* L. (onion), *Arachis hypogaea* L. (peanut), *Asparagus officinalis* L. var. *altilis* L. (asparagus), *Beta vulgaris* L. (beet, mangel), *Brassica oleracea* L. var. *botrytis* L. (cauliflower), *Bromus inermis* Leyss. (brome grass), *Capsicum frutescens* L. (pepper), *Cucumis melo* L. (muskmelon), *Cucumis sativus* L. (cucumber), *Hyoscyamus niger* L. (henbane), *Linum usitatissimum* L. (flax), *Lycopersicum esculentum* Mill. (tomato), *Pisum sativum* L. (pea), *Spinacia oleracea* L. (spinach), *Taraxacum kok-saghyz* Rod. (Russian dandelion), *Tetragonia expansa* Murr. (New Zealand spinach), *Trifolium hybridum* L. (alsike), and *Zea mays* L. (corn).

FUSARIUM OXYSPORUM Schlecht. emend. Snyder & Hansen form *lini* (Bolley) Snyder & Hansen

Snyder and Hansen, Am. J. Botany, 27 : 66. 1940.
Syn. *F. lini* Bolley

Wollenweber, Fusarium-Monographie, pp. 406–407. 1931.
Wollenweber and Reinking, Die Fusarien, p. 113. 1935.

This form of *F. oxysporum* was isolated from the seed of *Linum usitatissimum* L. (flax).

Section Martiella Wr.

Wollenweber, Phytopath. 3 : 30. 1913.
Wollenweber and Reinking, Die Fusarien, pp. 127–129. 1935.
Snyder and Hansen, Am. J. Botany, 28 : 738–740. 1941.
Gordon, Can. J. Botany, 30 : 238. 1952.

FUSARIUM SOLANI (Mart.) App. & Wr. emend. Snyder & Hansen
Wollenweber and Reinking, Die Fusarien, p. 135. 1935.
Wollenweber, Fusarium-Monographie II, pp. 181–182. 1944–45.
Snyder and Hansen, Am. J. Botany, 28 : 740. 1941.
Snyder and Hansen, Am. J. Botany, 32 : 662. 1945.
Gordon, Can. J. Botany, 30 : 239. 1952.

This species was isolated from the seed of *Aleurites fordii* Hemsl. (tung oil tree), *Allium cepa* L. (onion), and *Taraxacum kok-saghyz* Rod. (Russian dandelion).

Distribution of Species by Region and Host

Earlier in this paper it was indicated that most of the seed samples that were examined for the presence of species of *Fusarium* were from stocks grown in Canada, although some originated from stocks grown in other countries. More specific data are presented now with respect to the geographic origin of the seed of each host from which each species of *Fusarium* was isolated. Samples of seed from Canada are referred to by the name of the province from which they were obtained; those from the United States by the name of the state. Samples of seed from other countries are referred to only by the name of the country of origin.

Agropyron cristatum (L.) Beauv. (crested wheat grass)	
F. acuminatum	Alberta; Saskatchewan
F. sambucinum var. *coeruleum*	Saskatchewan
Agropyron trachycaulum (Link) Malte (Western rye grass)	
F. culmorum	Manitoba
Aleurites fordii Hemsl. (tung oil tree)	
F. solani	Source unknown
Allium cepa L. (onion)	
F. acuminatum	British Columbia; Manitoba
F. avenaceum	British Columbia
F. equiseti	Manitoba
F. moniliforme	Connecticut, Michigan, U.S.A.; Ontario
F. oxysporum	Manitoba; Ontario
F. solani	Ontario
Apium graveolens L. var. *dulce* DC. (celery)	
F. acuminatum	California, U.S.A.
F. equiseti	New York, U.S.A.; Ontario
F. poae	Ontario

Arachis hypogaea L. (peanut)	
F. compactum, F. equiseti, F. oxysporum	Source unknown
Asparagus officinalis L. var. *altilis* L. (asparagus)	
F. avenaceum, F. equiseti, F. oxysporum	British Columbia
Beta vulgaris L. (beet (*a*); sugar beet (*b*); mangel (*c*))	
F. acuminatum	Manitoba (*a*)
F. avenaceum	Quebec (*c*); Prince Edward Island (*c*)
F. culmorum	British Columbia (*b*)
F. equiseti	Manitoba (*a*); Quebec (*c*)
F. oxysporum	Ontario (*c*); Michigan, U.S.A. (*a*)
F. poae	Manitoba (*a*)
F. sambucinum var. *coeruleum*	Prince Edward Island (*c*)
Brassica napobrassica Mill. (Swede)	
F. avenaceum, F. equiseti, F. sambucinum var. *coeruleum*	New Brunswick
Brassica oleracea L. var. *capitata* L. (cabbage)	
F. equiseti	California, U.S.A.
Brassica oleracea L. var. *botrytis* L. (cauliflower)	
F. equiseti, F. oxysporum, F. sambucinum	British Columbia
Bromus inermis Leyss. (brome grass)	
F. acuminatum, F. culmorum	Saskatchewan
F. equiseti, F. oxysporum	Ontario
F. poae	Alberta
Capsicum frutescens L. (pepper)	
F. oxysporum	Michigan, New Jersey, U.S.A.
Carthamus tinctorius L. (safflower)	
F. acuminatum	Alberta
Cucumis melo L. (muskmelon)	
F. equiseti, F. oxysporum, F. sambucinum	California, U.S.A.
Cucumis sativus L. (cucumber)	
F. oxysporum	British Columbia; Nebraska, U.S.A.
Cucurbita pepo L. (pumpkin (*a*); vegetable marrow (*b*))	
F. equiseti	British Columbia (*b*); Ontario (*a*); Nebraska, U.S.A. (*a*)
F. sporotrichioides	British Columbia (*a*)
F. sambucinum var. *coeruleum*	British Columbia (*b*)
Cucurbita maxima Duchesne (squash)	
F. avenaceum, F. equiseti	British Columbia
Daucus carota L. var. *sativa* DC. (carrot)	
F. acuminatum	Manitoba
F. avenaceum	Denmark
F. culmorum	British Columbia
F. equiseti, F. poae	Ontario
F. sambucinum	Denmark
Festuca elatior L. (meadow fescue)	
F. equiseti	Ontario
Festuca rubra L. (red fescue)	
F. acuminatum, F. equiseti	Ontario
Glycine max Merr. (soybean)	
F. equiseti	British Columbia; Ontario; Quebec
F. moniliforme	Ontario
F. poae	Ontario; Quebec

Hibiscus esculentus L. (okra)	
F. equiseti	Michigan, U.S.A.
Hyoscyamus niger L. (henbane)	
F. equiseti, F. oxysporum, F. sambucinum	Ontario
Linum usitatissimum L. (flax)	
F. acuminatum	Saskatchewan
F. avenaceum	Ontario; Quebec
F. culmorum	Saskatchewan
F. equiseti	Ontario
F. graminearum	Quebec
F. oxysporum	Ontario
F. oxysporum f. *lini*	Saskatchewan
F. poae	Ontario
Lolium perenne L. (rye grass)	
F. arthrosporioides	Ontario
F. avenaceum	Scotland
F. culmorum	British Columbia
F. equiseti	Ontario
Lycopersicum esculentum Mill. (tomato)	
F. oxysporum	Ontario
Medicago sativa L. (alfalfa)	
F. avenaceum	British Columbia
F. equiseti	Ontario
Nicotiana tabacum L. (tobacco)	
F. poae	Ontario
Panicum miliaceum L. (millet)	
F. equiseti, F. poae	Ontario
Pastinaca sativa L. (parsnip)	
F. equiseti, F. poae	Prince Edward Island
Phaseolus coccineus L. (scarlet runner bean)	
F. culmorum	British Columbia
Phaseolus vulgaris L. (bean)	
F. acuminatum	Manitoba; Ontario
F. culmorum	British Columbia
F. equiseti	British Columbia; Manitoba; Ontario
F. poae	Manitoba
Phleum pratense L. (timothy)	
F. equiseti	Ontario
Pisum sativum L. (pea)	
F. acuminatum	Alberta; Ontario
F. avenaceum	Ontario; Quebec
F. culmorum	British Columbia
F. equiseti	Alberta; Ontario
F. graminearum, F. moniliforme	Ontario
F. oxysporum	British Columbia; Saskatchewan; Ontario
F. poae	Ontario; Quebec
F. semitectum	Quebec
F. sporotrichioides	Ontario
Raphanus sativus L. (radish)	
F. avenaceum	Holland
F. equiseti	British Columbia
F. poae	Ontario
Secale cereale L. (rye)	
F. acuminatum, F. poae	Manitoba
F. avenaceum	Quebec

Spinacia oleracea L. (spinach)	
F. acuminatum	Ontario
F. avenaceum	British Columbia; Holland
F. culmorum	Denmark; Holland
F. equiseti	Michigan, U.S.A.; England; Denmark; Holland
F. oxysporum	Quebec
F. poae	Ontario
Taraxacum kok-saghyz Rod. (Russian dandelion)	
F. acuminatum	Alberta
F. avenaceum	U.S.S.R.
F. equiseti	Montana, U.S.A.; U.S.S.R.
F. oxysporum	British Columbia; Minnesota, U.S.A.
F. sambucinum	Montana, U.S.A.
F. solani	Alberta
Tetragonia expansa Murr. (New Zealand spinach)	
F. equiseti, F. oxysporum	Ontario
Trifolium hybridum L. (alsike)	
F. acuminatum	British Columbia; Ontario
F. avenaceum, F. equiseti, F. moniliforme	Ontario
F. oxysporum	Alberta
F. poae	Ontario
Trifolium pratense L. (red clover)	
F. avenaceum	British Columbia
F. equiseti	Ontario; Quebec
F. graminearum	Ontario
F. poae	Manitoba; Quebec
Vicia faba L. (broad bean)	
F. acuminatum, F. equiseti, F. poae	British Columbia
Zea mays L. (corn)	
F. acuminatum	Manitoba
F. equiseti	British Columbia; Manitoba
F. graminearum	Ontario
F. moniliforme	Ontario; Connecticut, Colorado, Minnesota, Nebraska, U.S.A.
F. oxysporum	Manitoba; Ontario
F. poae	Manitoba; Ontario; Colorado, U.S.A.

Discussion

The results of the present taxonomic study have shown that an appreciable number of species, varieties, and forms of *Fusarium* are seed-borne by certain vegetable, forage, and miscellaneous crops. The majority of these fungi have not been previously reported to be seed-borne on the various cultivated plants from which they were isolated. Of the total of 16 species, varieties, and forms that were isolated from seed during this study, only four, namely, *F. moniliforme* and *F. graminearum* from the seed of *Zea mays*; *F. oxysporum* f. *lini* from *Linum usitatissimum*; and *F. avenaceum* from *Secale cereale*, were recorded by Orton (3) in 1931, in his bibliography of seed-borne parasites. A more recent tabulation by Porter (4) of the seed-borne species of *Fusarium* recorded in the literature shows that *F. avenaceum* has been isolated as well from the seed of *Daucus carota* var. *sativa*, and *F. oxysporum* from the seed of *Arachis hypogaea*.

That certain species of *Fusarium* appear to be more widely seed-borne than others is indicated by the number of different species of cultivated plants from which they have been isolated from the seed. Among those which appear to be rather widely seed-borne are *F. equiseti* (by the seed of 34 species of host plants); *F. poae* and *F. oxysporum* (by 18 species each); *F. acuminatum* (by 17 species); and *F. avenaceum* (by 16 species). On the other hand, certain species appear to be rarely seed-borne, namely, *F. sporotrichioides* (by the seed of two species of host plants), and *F. arthrosporioides*, *F. semitectum*, and *F. compactum* (by one species each). Of the species that were found during this study to be seed-borne, *F. culmorum* (by the seed of 10 species of host plants); *F. sambucinum* and *F. moniliforme* (each by the seed of five species); *F. graminearum* and *F. sambucinum* var. *coeruleum* (each by the seed of four species); and *F. solani* (by three species) may be considered as being occasionally seed-borne. However, a single species, for instance, *F. moniliforme*, may be commonly seed-borne by a particular host plant, in this case *Zea mays*.

From our present knowledge of the pathogenic capabilities of the species, varieties, and forms of *Fusarium* that were isolated from seed of the various crops it is possible to appraise their potential importance to crop production. Species such as *F. poae*, *F. equiseti*, *F. acuminatum*, *F. sambucinum*, *F. sambucinum* var. *coeruleum*, *F. semitectum*, and *F. compactum* are generally weak pathogens. Their presence in seed, and dissemination by this means, is therefore relatively unimportant. The extent to which they are seed-borne probably depends on relatively high atmospheric humidity prevailing during the crop period rather than on their inherent pathogenicity. While humid conditions during the crop period may be conducive to seed infection by all of the species of *Fusarium* isolated, the occurrence in seed, for instance, of *F. oxysporum*, should not be overlooked. Although this species may exist as a saprophyte, numerous pathogenic strains of it are known to be capable of causing wilts of many vegetable and other crops. Even a small percentage of seed harboring a wilt-producing strain of this species, for instance, *F. oxysporum* f. *lini* by the seed of flax, may serve to disseminate this pathogenic strain into areas where it has not been previously encountered in the soil. Because of their recognized pathogenic capabilities, the detection in seed of such species as *F. avenaceum*, *F. culmorum*, *F. moniliforme*, *F. graminearum*, *F. solani*, *F. sporotrichioides*, and *F. arthrosporioides* merits attention particularly if the species are known to cause, or are suspected of causing, disease of the crops by which they are seed-borne.

The results of this study, as well as those of a previous study (1), indicate that most species that occur more or less commonly in the soil or in other habitats in localities where seed is produced may be encountered in seed if the atmospheric conditions are relatively moist. Efficient seed treatment, however, should serve to prevent damage to seedlings that arise from infected seed, if pathogenic strains should be present, and at the same time prevent their effective dissemination by seed through the usual channels of commerce.

Acknowledgments

The writer is indebted to Margaret E. Lathe who assisted with the culture work and the preparation of the manuscript, and to Mr. W. E. Clark, who photographed the cultures and drawings.

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MICROBIOLOGICAL STUDIES OF AIR MASSES OVER MONTREAL DURING 1950 AND 1951[1]

By C. D. Kelly[2] and S. M. Pady[3]

Abstract

The numbers of bacteria and fungi in air masses over Montreal, Canada, were determined by sampling at the top of the Sun Life Building, 400 ft. above ground level, from September 1950 to December 1951. During 113 days of sampling, 978 exposures were made with agar plates in the General Electric Bacterial Air Sampler and the Bourdillon Slit Sampler. Eighty-seven exposures for fungus spores were made with silicone coated slides in the Slit Sampler. Most of the air encountered was continental polar either moving direct to Montreal, found on 38 days, or modified by moving over agricultural land, sampled on 51 days. Maritime polar air from the Atlantic was encountered on 21 days, and from the Pacific on one day. Maritime tropic air from the Gulf of Mexico was encountered twice. The seasonal variation of bacteria and fungi in all air masses showed low counts in January, February, and March. Higher bacterial counts were obtained from early April into July and from early September into November, covering a range from 3.0 to 55.0 per cu. ft. Lower counts were found during late July and August. The high fungus counts extended from May into November and covered a range from 5.0 to 56.3 per cu. ft. Both continental polar and maritime polar air that moved over considerable cultivated land showed generally higher counts than where these air masses moved directly to Montreal.

During the summer of 1947 studies on bacteria and fungi in arctic air were carried out by exposing Petri plates and vaseline coated slides from slow moving aircraft (12, 9, 10). Further investigations were made in the Arctic during 1949 and 1950, using quantitative methods (7, 11). Sampling from aircraft did not always prove satisfactory, as it permitted taking only a limited number of samples covering a wide area and was restricted to the air masses encountered on a particular flight. Therefore it was concluded that by taking samples at a fixed high point, many more samplings could be made and a greater variety of air examined. Following this, samples were taken during the summer of 1950 at the top of the hangar at Fort Churchill, Man. (7). From these and further studies over non-arctic regions (2, 8), it was apparent that microorganisms carried in arctic air were largely the residue of organisms picked up from soil in more temperate zones and carried north by air masses. The project was therefore directed towards the microbiological study of air masses in general. The data reported in this paper are from samples obtained between September 1950 and December 1951 from the top of the Sun Life Building in Montreal, Que.

[1] *Manuscript received May 21, 1954.*

Contribution from the Bacteriology and Botany Departments, McGill University, Montreal, Quebec. The work was supported by a grant (No. 175) from the Defence Research Board, Ottawa, Canada. The co-operation of the Sun Life Assurance Company of Canada is gratefully acknowledged. We would like to express our appreciation of the invaluable technical assistance of Lucia Kapica and Sharon Layne.

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Methods

The methods used in this investigation have been described in previous publications (3, 7, 6). The General Electric Electrostatic Bacterial Air Sampler (5) and Bourdillon's Slit Sampler (1) were operated upon a table placed at the windward side of the roof of the Sun Life Building. When in operation the samplers were 400 ft. above ground level. The amount of air sampled varied from 2 to 20 cu. ft. depending on the anticipated number of organisms. Agar plates were used in both samplers for viable organisms, and silicone coated slides for total fungus spore count were exposed in the slit sampler with the turntable held stationary and the slide laid lengthwise under the slit (6). The media used were Czapek's agar modified by adding 0.1% yeast extract and 0.5% glucose (9), and Lochhead's yeast extract agar (Medium Y) (4). The plates were incubated at 25° C. and counted at the end of five days.

In all, 978 separate exposures were made with agar plates during 113 days of sampling, and each daily average was calculated from one to ten samples. Though Czapek's and Lochhead's media were both used throughout the whole period of sampling, the results were sufficiently close that they have been used together in obtaining these averages. Eighty-seven silicone coated slides were exposed in the slit sampler.

Air Masses

Air masses present over Montreal at the time of sampling were plotted and their history determined as far as possible.* The bacteria and fungi found in air are largely soil forms swept up by air moving over agricultural land. In time, with no fresh contamination, the numbers of these are reduced by death of the organisms or precipitation by rain, hail, and snow. With this in mind it would seem that the number of bacteria and fungi would provide further information on the history of an air mass. As the number of organisms in an air mass should bear some relationship to the time spent over cultivated land, the air masses are further classified to show the amount of contact with agricultural areas. By far the most common type of air was continental polar (cP), with maritime polar (mP) next. Twice maritime tropic air (mT) from the Gulf of Mexico was encountered.

The continental polar air has been divided into five subgroups on the amount of exposure to agricultural land: cP1, fresh polar air direct from the Arctic; cP2, polar air that has become stagnant over the Montreal area; cP3, continental polar air that had moved for a short distance over agricultural land; cP4, continental polar air that had moved a considerable distance over agricultural land; cP5, continental polar air that moved from the Pacific coast area over the northern United States. The air from the Atlantic Ocean was all maritime polar air and was classified into three subgroups: mP1, air that came in over Labrador and had little or no contact with cultivated land;

* *The assistance of Mr. Sven Orvig, of the Arctic Institute in Montreal, in working out the history of the air masses is gratefully acknowledged.*

mP2, Atlantic air coming to Montreal over the part of the coast extending from the mouth of the St. Lawrence river to New Jersey; mP3, air that came in over the coast of Virginia or the Carolinas and passed over a considerable area of farm land; maritime polar air that came from the Pacific and moved to Montreal by way of southern Canada was encountered once and was called mP4. The air from the Gulf of Mexico was maritime tropic (mT).

Results

The numbers of bacteria and fungi per cubic foot have been determined for each sampling method and combined daily averages from the two media are recorded in Table I. In comparing the two media, the bacterial counts from Lochhead's medium were higher than from Czapek's in 66.6% of cases, and with the fungi, 57.5% of the counts were higher with Czapek's medium. However, the difference in numbers of organisms on the two media was not great except in some cases where the number of bacteria on Lochhead's medium was much higher than on Czapek's medium. It was noted with Lochhead's medium that the fungus colonies tended to be smaller and more difficult to identify than those on Czapek's medium.

Duplicate samples were taken with the General Electric and slit samplers excepting when the slit sampler was used to expose slides and during the time when the slit sampler was used on two flights to England (8) (Table I). Out of 92 daily averages from such samples, 85 bacterial and 61 fungus counts were higher with the General Electric sampler. The numbers of bacteria on six days and the numbers of fungi on 28 days were higher with the slit sampler, while with the remainder the numbers were the same (bacteria on one day and fungi on three days). Throughout this paper where counts of bacteria and fungi are mentioned they are from the G. E. sampler unless otherwise stated. A great many more agar plates were exposed in this sampler than with the slit sampler, as it was used continuously throughout the whole period, while the slit sampler was used at times for fungus spore counts and on two flights over the Atlantic as mentioned above.

Counts of bacteria and fungi are recorded in Table I, where the influence of the seasons may be observed. During the period of January, February, and March, when the surface of the soil is frozen and activity of microorganisms is at a minimum, counts of microorganisms in air samples were very low. The numbers of bacteria in all air masses encountered exceeded 2.0 per cu. ft. on only 2 of 21 days, while the fungi had but one count over 2.0 per cu. ft. and one over 1.0. The bacteria as a rule were present in greater numbers than fungi during this period.

As found in earlier studies (2), the bacteria in the air were in greater numbers in spring and fall than during the summer, though in Montreal the period of high numbers tended to be later. There was a steady rise in numbers of bacteria in continental polar air masses from below 2.0 per cu. ft. in March to a high on June 21 of 20.4 per cu. ft., followed by a steady drop to the end of August. There was a further rise from the end of August to a high point

TABLE I

SUMMARY OF DATA ON DAILY AVERAGE NUMBER OF ORGANISMS PER CUBIC FOOT IN AIR SAMPLES TAKEN ON TOP OF THE SUN LIFE BUILDING

Date	Air mass*	G. E. sampler			Slit sampler			Slides in slit sampler	
		No. samp.	Bac. per cu. ft.	Fungi per cu. ft.	No. samp.	Bac. per cu. ft.	Fungi per cu. ft.	No. samp.	Fungus spores per cu. ft.
1950									
Sept. 22	cP3	10	7.9	5.1					
Oct. 6	cP3	6	14.0	6.4					
20	cP3	8	9.4	7.9					
30	cP5	4	8.4	25.4				2	141.1
31	cP1	4	14.6	6.0	4	6.4	6.5	4	46.6
Nov. 1	cP1	6	22.1	11.1	4	6.7	5.8	2	
2	mT	2	32.9	38.0	1		10.0	1	320.0
3	mP4	4	27.3	6.9	4	9.1	5.1	4	36.0
6	cP1	6	21.9	3.1	4	9.9	2.9	3	22.3
21	cP1	3	11.6	5.1	2	8.1	3.2	2	14.2
22	cP1	7	26.8	2.4	4	7.6	3.4	4	5.8
23	cP3	6	55.0	6.0	4	24.5	2.4	3	88.1
24	cP3	7	35.8	4.6	4	14.5	3.7	2	9.8
27	cP4	6	9.6	1.1	4	5.8	1.9	2	6.6
28	cP3	5	7.9	2.0	4	4.5	2.8	1	2.0
29	cP3	6	2.1	1.3	4	2.2	1.8	2	2.7
30	cP3	3	5.1	2.8	2	4.1	1.9	2	2.8
Dec. 1	cP1	4	12.7	2.0	3	4.7	1.3	1	0.8
1951									
Jan. 2	cP5	5	1.6	0.4	4	0.8	0.2	1	5.3
3	cP5	5	1.3	0.3	4	2.2	0.4	2	4.0
4	cP1	5	2.9	1.4	4	0.8	0.6	2	9.6
5	cP1	6	5.7	1.1	4	2.3	1.0	1	1.3
8	cP1	6	1.1	0.2	4	0.7	0.7	2	0.8
10	cP5	5	0.4	0.9	4	0.1	0.7	2	8.2
22	cP1	3	0.7	0.3					
23	cP3	3	0.6	0.4	2	0.2	1.1	1	0.2
24	cP5	5	1.5	0.1	4	1.0	0.3	2	2.5
Feb. 6	cP1	5	0.4	0.5	4	0.2	0.2	2	1.5
7	cP5	1	0.1	0.0					
19	mP2	5	0.6	0.1	4	0.3	0.1	3	3.8
20	mP2	5	0.7	0.6	4	0.2	0.5	1	3.0
21	mP2	5	1.9	0.5	4	0.4	0.5	1	6.0
22	mP2	4	0.5	0.2	4	0.3	0.3		
23	cP1	4	0.8	0.4	4	0.6	0.5		
Mar. 12	cP1	5	1.1	2.6	4	0.4	0.9	2	2.6
13	cP3	5	1.2	1.6	4	1.0	1.0	1	0.4
14	cP3	5	1.9	0.8	4	0.3	0.3	2	1.6
15	cP4	4	0.8	0.6	2	1.2	0.9	1	0.0
16	cP4	5	1.6	1.3	4	0.6	1.1	1	19.0
Apr. 9	cP3	4	4.6	2.6	3	2.8	1.9	2	8.8
10	mP2	5	21.7	3.9	4	6.8	3.6	1	3.0
11	mP2	5	12.5	5.0	4	4.1	3.7	1	4.0
12	mP2	5	7.3	3.5	4	3.6	4.5	2	11.3
13	cP4	5	1.7	3.1	4	1.3	3.6	2	2.8
23	cP3	5	7.5	2.3	4	4.0	2.6	2	14.0
24	cP1	5	4.3	1.5	4	2.6	2.1	2	6.6
25	cP1	5	3.7	3.2	4	0.4	2.7	2	10.6
26	cP3	5	4.9	1.9	4	1.9	2.3	2	14.3
27	cP2	4	10.2	4.6	2	0.1	1.1	1	61.0

** Air masses: cP1, fresh continental polar air; cP2, continental polar air stagnant over Montreal; cP3, continental polar air modified by short exposure over agricultural land; cP4, continental polar air modified by a longer exposure over agricultural land; cP5, continental polar air that moved from the west to the east over the United States; mP1, fresh maritime polar air from the Atlantic; mP2, maritime air from the Atlantic that moved in over the coast between the mouth of the St. Lawrence River and New Jersey; mP3, maritime polar air that came in south of New Jersey; mP4, maritime polar air from the Pacific that moved over southern Canada; mT, maritime tropic air from the Gulf of Mexico.*

TABLE I—*Concluded*

SUMMARY OF DATA ON DAILY AVERAGE NUMBER OF ORGANISMS PER CUBIC FOOT IN AIR SAMPLES TAKEN ON TOP OF THE SUN LIFE BUILDING—*Concluded*

Date	Air mass*	G. E. sampler			Slit sampler			Slides in slit sampler	
		No. samp.	Bac. per cu. ft.	Fungi per cu. ft.	No. samp.	Bac. per cu. ft.	Fungi per cu. ft.	No. samp.	Fungus spores per cu. ft.
1951									
May 7	cP1	5	7.6	3.2	4	2.5	2.8	2	17.4
8	cP1	5	7.9	3.5	4	2.2	2.8		
9	cP1	5	8.6	5.4	4	2.7	3.3	1	21.2
10	cP1	5	5.5	2.6	4	1.7	1.6	1	4.6
11	cP2	5	8.6	6.5	4	4.8	4.6	1	73.0
21	cP2	5	7.9	6.2	4	1.6	3.2	1	78.4
22	cP2	5	13.8	10.1	4	2.8	6.8	1	111.2
23	mP1	4	5.2	7.7	4	1.5	4.3	1	67.6
24	mP1	4	5.5	8.3	4	1.9	6.2	1	70.8
25	mP1	5	10.8	7.9	4	3.1	4.7	1	116.0
June 11	cP1	5	8.8	6.4	4	2.8	3.4	1	21.6
12	cP2	5	15.9	14.8	4	6.2	10.5		
13	cP2	5	8.3	11.7	4	2.0	8.2	1	73.0
14	mP3	5	3.7	10.3	4	2.7	3.4	1	45.6
15	mP3	5	9.6	11.4	4	5.2	5.1	1	214.9
18	mP2	1	4.5	10.1					
19	mP3	2	10.7	20.8					
20	mP3	2	4.5	56.3					
21	cP3	2	20.4	41.4					
22	cP1	2	6.4	19.2					
25	cP1	2	11.8	10.9					
26	cP1	2	2.3	7.0					
27	cP3	1	10.2	28.4					
28	cP2	2	7.5	11.1					
29	cP3	1	7.8	9.6					
July 19	cP1	5	2.5	7.3	4	1.1	3.0	1	227.2
20	cP1	5	10.1	30.5	4	5.8	26.2	1	194.4
23	cP1	6	3.3	11.7	5	1.2	8.1	1	364.0
24	cP2	5	7.8	14.5	4	1.3	11.8	1	192.2
25	cP2	5	6.1	22.9	4	1.1	15.8	1	328.0
26	mT	5	5.1	25.7	4	2.2	18.6	1	229.0
27	cP1	6	4.0	12.4	5	1.2	8.0	1	173.2
Aug. 15	cP1	5	3.5	14.0	4	1.9	21.8	1	200.4
16	cP1	6	5.1	23.9	4	1.8	27.4	1	351.9
17	cP1	5	4.2	9.1	4	4.6	8.1	1	33.6
20	cP2	2	3.1	15.7					
22	cP3	2	16.8	32.1					
23	cP1	2	2.1	10.7					
24	cP1	2	4.0	7.8					
Sept. 4	cP1	4	2.1	22.0	4	2.1	24.6		
5	cP1	6	4.2	9.4	4	2.6	13.2	1	94.6
6	cP2	5	15.4	31.1	3	8.1	23.9	1	445.0
7	cP1	5	1.4	13.9	4	2.3	4.1	1	205.0
17	cP2	5	3.1	5.9	4	1.4	6.0	1	58.2
18	cP1	5	5.0	7.6	4	1.3	6.3	1	64.0
19	cP4	5	2.6	13.5	4	1.5	14.2	1	181.6
20	cP1	5	5.8	11.6	4	2.2	4.9	1	174.0
21	cP4	10	5.3	26.0	7	3.0	23.1	2	105.1
Oct. 1	cP4	5	9.3	9.4	4	4.1	5.9	1	33.0
2	cP2	5	4.1	17.6	4	2.1	14.6	1	19.0
3	cP2	4	7.2	18.3	4	4.5	9.0	1	110.6
4	mP3	4	4.4	10.8	4	4.2	18.7	1	57.7
5	cP1	4	4.1	27.5	4	1.2	20.3	1	184.0
15	mP3	5	4.5	10.0	4	1.7	10.3	1	16.4
16	mP3	4	3.8	4.8	4	1.4	5.2	1	22.4
17	mP3	3	4.0	14.9	2	1.1	7.3	1	12.8
18	mP2	3	8.5	11.4	2	3.7	9.4	1	17.9
19	mP2	3	3.7	21.7	2	1.1	24.0	1	45.4
Nov. 5	cP4	3	7.2	3.5	2	1.7	3.7	1	1.2
6	cP4	3	7.0	5.4	2	4.1	4.1	1	15.0
8	cP4	2	1.4	2.2	2	1.2	2.3	1	2.8
26	cP3	3	0.3	3.3	2	0.2	2.0	1	4.0
30	cP4	3	10.3	10.0	2	4.1	4.1		
Dec. 19	cP3	1	1.0	0.6	2	1.5	0.6	1	59.3
TOTAL		507			344			127	

in November (55.0 per cu. ft. November 1950 and 10.3 November 1951) and then a rapid fall to the winter low. In maritime polar air the seasonal results were more irregular.

The fungi also showed a seasonal pattern in all air masses similar to that found previously in air where the numbers reached a maximum during August (2). The first increase in numbers of fungi was noted early in May and high counts were found until the end of October. The highest plate count (56.3 per cu. ft.) was found in June and the lowest (4.8 per cu. ft.) in October. During this period, only 9 out of 47 average counts were under 10.0 while 14 were over 20.0 per cu. ft. The numbers of fungi were consistently higher than the bacteria during this time and much higher than the average winter counts of 0.0 to 2.5 per cu. ft.

Fungus spore counts from silicone treated slides, exposed in the slit sampler, showed similar trends to the numbers from plate counts, except that the numbers were usually much higher. In winter when numbers were low, plate and slide counts were at times the same but sometimes slide counts were 20 times higher. During the summer all counts were 10 to 15 times higher and in two cases, 30 and 40 times greater than corresponding plate counts. During winter numbers of fungus spores varied from 0.0 to 10.0 per cu. ft. (Table I). From March to June there was a steady increase with very high numbers frequently occurring during the summer, when numbers of 364, 351, and 445 fungus spores per cu. ft. were recorded (Table I).

The highest bacterial counts were 55.0 and 25.8 per cu. ft. obtained during November 1950 from modified polar air (cP3) that had moved down from the Yukon, over northern Saskatchewan and Manitoba, then over the Great Lakes and New York State to Montreal. For the fungi the highest plate count was 56.3 per cu. ft. on June 20 from maritime air (mP3) that had come in over the Carolinas and north through West Virginia, Pennsylvania, and New York State. The following day (June 21) a count of 41.4 was found in modified polar air (cP3) that had come to Montreal by way of Hudson Bay, the Great Lakes, and southwestern Ontario. On November 2, 1950, a fungus plate count of 38.0 per cu. ft. was found in maritime tropical (mT) air that had come north from the Gulf of Mexico. The fungus spore count at the same time was 320 per cu. ft. The highest readings of fungus spores were found in continental polar air, where 445 per cu. ft. were obtained in cP2 air September 10 and 364 per cu. ft. in cP1 air July 23.

During January, February, and March bacterial counts in all types of air examined were consistently low. At the time of highest counts, the number of bacteria in pure polar (cP1) air ranged from 4.1 to 26.8 per cu. ft. during October and November and 2.3 to 11.8 during May, June, and July (Table I). In modified polar air, during October and November counts ranged from 0.3 to 55.0 per cu. ft. and from 6.1 to 20.4 in May, June, and July. Because of seasonal and other variations, it was difficult to make comparisons of the load of microorganisms in the various air masses, but the four types of modified continental polar air (cP2, cP3, cP4, cP5) tended to have higher bacterial counts than the cP1 polar air.

Numbers of fungi in the various types of continental polar air showed considerable variation. In the period of highest counts from the end of May to the end of November, fresh polar air (cP1) had counts ranging from 6.4 to 30.5 per cu. ft. In modified polar air, during this same time, the lowest was 5.8 per cu. ft. in cP3 air on September 17 and the highest 41.4 in cP3 air on June 21. Modified polar air tended to carry greater numbers of fungi than the pure polar air.

Maritime air from the Atlantic, the next most common type encountered, was found at intervals during February, through the summer, and into October and was all maritime polar. In February the counts for both bacteria and fungi were low (0.1 to 1.9 per cu. ft.) with the bacterial counts slightly higher than the fungus. In April the bacterial counts in Atlantic air that came in over Newfoundland and southeastern Quebec (mP2) showed an increase (21.7, 12.5, 7.3 per cu. ft.), while the fungus count remained below 5.0 per cu. ft. In May from air that came in over Labrador (mP1), counts for both bacteria and fungi varied from 5.2 to 10.8 per cu. ft. Maritime air that came in over the Carolinas (mP3) in June carried about the same number of bacteria as the mP2 air found in May but the fungus count varied from 10.3 to 56.3 per cu. ft. During October maritime air from the Carolinas (mP3) was sampled on four days and on two days similar air from New England (mP2) was encountered. The bacterial count from these samples varied from 3.7 to 8.5 per cu. ft. while the fungus count varied from 10.0 to 21.7. One air mass of maritime polar air from the Pacific (mP4) was encountered in November and gave average counts of 27.3 bacteria and 6.9 fungi per cu. ft. Air from the Gulf of Mexico (mT) was encountered on one day in November with a content of 32.9 bacteria and 38.0 fungi per cu. ft. and one in July with 5.1 bacteria and 25.7 fungi. High counts of fungus spores were obtained frequently between May and November. Loads in excess of 100 per cu. ft. were recorded on 21 occasions and at least once each month during this period. The maximum reading of 445 spores per cu. ft. recorded on September 6 was in cP2 air (Table I); other high readings were 364 on July 23 in cP1 air, 351.9 on August 16 in cP1 air, and 320 per cu. ft. on November 2, 1950, in air from the Gulf of Mexico.

Details on the kinds of fungi obtained on the silicone slides as well as fungi and bacteria found on the agar plates will be published separately.

Discussion

During 1950 and 1951 air samples for bacteria and fungi were taken at the top of a high building in Montreal, 400 ft. above ground level. This sampling covered a period of 16 months and represents a series of 978 individual samples taken on 113 days. Taking samples from a fixed high point in close proximity to McGill University laboratories made it possible to obtain and study much more material than when samples were taken from aircraft. Such samples gave a comparison of the load of organisms in various types of air masses and show the effect of the seasons on the numbers and types of organisms.

In this study, the General Electric and slit samplers were both used to obtain viable counts of bacteria and fungi. Though the counts from the two samplers were relative, the General Electric sampler gave higher counts in the majority of cases.

The use of silicone coated slides in the slit sampler for making total fungus spore counts is a distinct advantage. This is especially true, since it gives, in conjunction with the viable plate count, an estimation of the number of nonviable spores. Also the numbers of viable and nonviable spores together with the types of spores provide information on the history of the air mass. Some arctic and maritime air carried quantities of nonviable spores that could only come from agricultural land and had been in the air long enough to die from exposure. By sampling throughout the year at a fixed high point well south of the arctic, it was expected that both arctic and tropical air would be encountered on a sufficient number of days to make possible a satisfactory comparison of the number of bacteria and fungi in these two extreme types of air. Through lack of a sufficient number of samples of tropical air such a comparison could not be made, as the samples were largely from air of arctic origin and only two were of tropical air.

As the bacteria and fungi in the outer air are soil bacteria and fungi, the various air masses encountered were classified on the basis of the amount of contact with cultivated land where fresh organisms might be swept into the air. Higher counts would therefore be expected from air that had been for some time over agricultural areas than from pure arctic or maritime air. In the main this was found to be true, for in most cases, where comparisons could be made, lower counts were obtained from pure arctic or pure maritime air than from similar air masses that had been modified by passing over farm land.

In a few cases high or low counts were encountered where the opposite would be expected. Low counts of air from over cultivated land could be explained by the organisms being washed out of the air by rain. An example of this is seen with the fungus counts of June 14 and 15 in maritime air that had moved up through the Carolinas, West Virginia, Pennsylvania, and New York State. During this period there were scattered showers over the whole area and the air at Montreal carried 11.7 and 10.3 fungi per cu. ft. Similar air, without showers, on June 19 and 20 carried 20.8 and 56.3 fungi per cu. ft. respectively.

On the other hand it is difficult to understand why pure arctic air (cP1), which had not come in contact with cultivated land on the way to Montreal and would be expected to carry low numbers of organisms, had at times viable counts of bacteria (Nov. 1950) and fungi (July, Aug., Sept., and Oct. 1951) exceeding 20 and 30 per cu. ft. Also during this study, slides exposed on nine days in continental polar air (cP1) that had moved from around Hudson Bay direct to Montreal had counts of fungus spores exceeding 100.0 per cu. ft. and ranged from 173.2 to 364.0. Counts of this magnitude were not encountered during earlier studies in the Hudson Bay area (7), excepting in

air that had moved from the south. These continental polar air masses were not traced beyond Hudson Bay and it would seem, from the number of micro-organisms, that the air had recently come north from agricultural areas.

As noted in previous studies (2), fungus counts during the winter months were low and never exceeded 2.0 per cu. ft. at any time. In continental and maritime polar air there was a slight rise in numbers in April and May, with high numbers from the first of June to the end of October, followed by a rapid drop through November to the winter low in December. High fungus counts were encountered in November 1950 in air from the Gulf of Mexico and from the Pacific across southern Canada. This period of high counts was during the time when the growth of fungi was most abundant and when the greatest number of spores were available for distribution by air.

In earlier studies (2) the bacteria were found in greatest numbers in March and October, with much lower counts in July and the lowest counts during the winter months. The picture was not complete however, as samples were not taken in the intervening months. Here with many more samples over the year, the trend in bacterial numbers was fairly clear. The lowest counts were found during the winter months to the end of March with a definite increase in April, especially in maritime air. The general trend showed a steady increase to June 21 and then, with the exception of one average count in August of 16.8 per cu. ft. in modified maritime air (mP3), counts for July and August were generally lower. There was a further rise in numbers starting in September to a high in November, followed by a rapid drop. This high point in November was much more pronounced in 1950 than in 1951.

One difficulty in a combined study of bacteria and fungi is to find a medium suitable for growing the greatest number of both organisms. Fortunately both groups are of soil origin and their nutritional requirements are not greatly different. A modification of Czapek's medium and a medium used by Lochhead for soil studies gave best results. Both bacteria and fungi grown on the two media showed little difference in numbers. With the fungi the Czapek's showed higher counts in 57.5% of cases, while for bacteria the Lochhead's medium had higher counts 66.6% of the time. The spread in counts between the two media was greater in the case of bacteria than fungi and when bacteria and fungi were involved for counts alone the Lochhead's medium would seem the most satisfactory. However, the fungus colonies on Lochhead's medium were smaller and more difficult to identify than those on Czapek's medium. Therefore when rapid identification of fungus spores is desired Czapek's medium would seem preferable.

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SOME PLANT MICROFOSSILS IMPORTANT TO PRE-CARBONIFEROUS STRATIGRAPHY AND CONTRIBUTING TO OUR KNOWLEDGE OF THE EARLY FLORAS[1]

By N. W. Radforth[2] and D. C. McGregor[3]

Abstract

Fifty-six recently discovered spores and sporelike microfossils from Canadian non-coaly deposits of Middle and Upper Devonian age are described and classified. Their manner of occurrence suggests their probable usefulness in the establishment of biostratigraphic entities, and their applicability to the stratigraphic study of oil-bearing rocks. Relative to the history of plants, the microfossils convey evidence of a more complex and more highly evolved Devonian flora than has been apparent from the macrofossil record. In addition, preliminary examination has disclosed spores of comparable abundance and of only slightly less complexity in rocks of Lower Devonian and Silurian age.

Introduction

In Canada it has become evident that rocks of Devonian age are associated with the occurrence of oil and gas. In some of these Devonian areas, prospecting has been difficult because of the presence of "unfossiliferous" sediments, the presence of a high degree of faciation,[4] or the lack of reference index material. In these situations, establishment of age and stratigraphic relations within the potentially productive strata should be assisted by any new agents or methods which become available. Of immediate aid to solution of these problems would be the discovery of applicable micropalaeobotanical evidence.

A further problem, one which is of particular import to the palaeobotanist, results from the limited floral material that has been obtained from Devonian rocks. Relative to the condition in the Carboniferous, the Devonian period has been regarded as disappointingly barren of floral remains. This is unfortunate, since it is widely accepted that this period is a critical one from the standpoint of the origin and differentiation of terrestrial plant life. There is an urgent need for a re-evaluation of the pre-Carboniferous flora, and the presentation of additional floral evidence would be of immediate value.

Considerable success has been achieved with Cretaceous and Carboniferous coals, toward the solution of both stratigraphic and botanical problems, through the use of fossil spores and pollens. Because of the small size and the resistant nature of these bodies, which well adapt them to preservation, and because of their suitability for uniform dispersion in large numbers, they are inherently appropriate for both problems mentioned above. Within

[1] *Manuscript received March 25, 1954.*
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[4] *Faciation in the geological sense, i.e., an abundance of bio- or litho-facies, rather than in the sense of the biological community of the ecologist.*

various coal matrices, and in the shales associated with some coals, plant microfossils have been utilized to demonstrate correlation by several workers (5, 7, 22, 23, 33). In addition, evaluations have been made of past floral assemblages on the basis of microfossils obtained from coal (9, 11, 29, 35).

Plant microfossils have been reported in varying degrees of abundance from shales and sandstones (17, 26, 27, 34, 36), although only to a relatively limited extent (especially for northern North America) in pre-Carboniferous sediments (15, 19, 25). Upon consideration of this evidence, and with the realization of megascopic plant remains in some early sandstones and shales, it has seemed reasonable to propose that further research would reveal perhaps even an extensive microfloral population in various pre-Carboniferous inorganic rocks.

The objectives of the problem of evaluation of the plant microfossil content of pre-Carboniferous rocks are detailed here by the nature of the problem as a preliminary study. Determinations must be made as to the existence of spores and pollens in various matrices. Difficulties of extraction and concentration must be anticipated, and suitable techniques must be derived for these processes. The extent to which microfossils from certain inorganic matrices may be utilized in stratigraphic correlation can then be evaluated. Finally, the degree of applicability of the disclosed units to the palaeobotany of the pre-Carboniferous periods is ascertainable. These four aspects provide the basis for the present investigation.

Selection of Material

Through the courtesy of Imperial Oil Limited, segments of a drill core were obtained from the Anglo-Canadian Wabamun Lake No. 1 well, a "dry hole" located in Section 10, Township 51, Range four, west of the fifth meridian, in west central Alberta. The stratum from which detailed study was made was a soft crumbly gray mudstone, representing the 6403-ft. depth of the core. The age of this level was designated on an electrolog as Upper Devonian. Other rock of Devonian age was obtained from L'Anse-à-Brillant, Douglas township, Gaspé, Que. This material was a portion of an outcrop of medium to fine grained slightly laminated gray-green sandstone, throughout which numerous small bright quartz crystals and larger (0.5 mm. to 5.0 mm.) black and often irregular bars of carbonaceous material were visible. The same rock also contained fragmentary stem compressions, some of which were included with the matrix in the macerations. The outcrop in the area from which this sample was taken has been classified as Middle Devonian Battery Point Formation (16).

Preliminary reference has also been made to Devonian rock samples from Abitibi River, southwest of James Bay, Ont. (Sextant formation) and to Silurian samples from Forillon Pen., Gaspé, and Charlotte Co., N.B.[5]

[5] *Supplied through the courtesy of Geological Survey of Canada, collection per L. M. Cumming.*

Treatment of Material

Modifications of the techniques described by Wilson (35), Kosanke (12), and Radforth and Rouse (22) were used to prepare the material for microscopic examination. The basic procedure consisted of immersion of the fragmented rock in (i) hydrofluoric acid, 48% (reagent quality), (ii) ammonium hydroxide (reagent quality), (iii) hydrochloric acid, 6 *M*, (iv) a modification of Schulz's reagent, consisting of concentrated nitric acid plus approximately ¼ cc. of potassium chlorate crystals, and (v) potassium hydroxide 10%, warmed to between 80° C. and 90° C. The sediments were centrifuged and washed before each solution change.

The hydrofluoric acid treatment was used to dissolve siliceous material, the minimum times required for this process being 47 hr. for the mudstone and 75 hr. for the sandstone. The times of immersion of the rock chips in ammonium hydroxide and hydrochloric acid, on the other hand, were apparently not specific, but one hour was allowed for each.

Oxidation of the organic material with nitric acid and potassium chlorate was the most specific process in time requirement. Periodically, small portions of the oxidizing mixture were washed with water and examined for microfossils. When spores were visible, the oxidizing agent was decanted and the sediment was carefully washed three times. All samples were then treated with warm potassium hydroxide for 10 min. In subsequent work with palaeozoic sandstones it has been found that the final alkali treatment may often be omitted.

The corn sirup slide medium described first by Radforth (21) and used by Radforth and Rouse (22) was employed in the present investigation, since it affords a fast and simple means of producing large numbers of slides.

The system of letter designation of spore types, upon which our microfossil key (p. 619, Appendix I) is based, is an adaptation of the system of letters and subscripts devised by Raistrick and Simpson (24).

The fact that a natural classification of fossil spores is impossible at the present time has been commented on by several workers (3, 11, 18, 20, 30). Meanwhile, each of these investigators has proposed a classification method which, although still largely artificial, employs a binominal nomenclature. Undoubtedly such systems offer most desirable means of organization and reference. However, in the present case it has been found more practical to use the letter system, primarily because of the relatively imperfect condition of many of the pre-Carboniferous spores. Inadequate preservation of haptotypic features and germinal furrows, coupled with varying degrees of corrosion of the spore walls during maceration, makes it undesirable to base a classification and nomenclature on the definite presence or position of these features. It is desirable, of course, that the newly discovered microfossils be recorded because of their usefulness in spite of occasional imperfections. When the true nature of the diagnostic structures of the various forms has been determined, the generic system of nomenclature may profitably be adopted.

Results

Each of the two strata has disclosed numerous spores[6] and spore-like bodies in varied conditions of fragmentation, as well as many pieces of cuticle and other organic particles which absorbed the safranin stain. All spores were compressed, usually in the transverse plane.

Of the total 91 spore types that were distinguished in these horizons, 56 of the best preserved are included in the present account. The authors have not demonstrated relationships of any of these bodies with spores described by other workers, although it has been noted in several instances that morphological similarities exist. On this basis the possible spore relationships and parent origins of some forms are suggested. It must be realized, of course, that similarities between spores of different geological age may or may not have phylogenetic significance, and that the separation of one from another in time or space lessens the probability that phylogenetic relationships exist (30, pp. 8-9). For any spore that displays a unique set of characters the phrase "not previously described" is appended.

Spore Descriptions, Wabamun Lake Sample

A_3—(*Fig. 1*)

Outline broadly oval in transverse plane, with the long axis of the microfossil at right angles to the long axis of the spore body. Air-sac thus narrowest opposite the narrower transverse axis of the body. Wall of air-sac with radial folds which extend out of plane of body, indicating position of attachment of this wing to be proximal or distal, or both. Haptotypic features, if present, obscured by folds parallel to the long axis of the spore. Diameter including air-sac, $38\mu \times 44\mu$; body dimensions $23\mu \times 32\mu$. Ornamentation of body and air-sac granulose.

This microfossil may be classified as *Zonalomonoradites* Pant (20) if a proximal monolete aperture is present. It is possibly allied to the Cordaitales or Coniferales. Not previously described.

B_7—(*Fig. 2*)

Body outline obovate. Air-sacs oval and broader than body, imparting a dumbell-like outline to the microfossil when compression is transverse.

[6] *The word "spore" is used here as a general term, and includes not only spores in the strict sense, but also prepollens and pollens, if these structures are present.*

FIG. 1. A_3 $38\times44\mu$.
FIG. 2. B_7 $30\times65\mu$.
FIG. 3. B_8 $56\times80\mu$.
FIG. 4. B_{12} $35\times52.5\mu$.
FIG. 5. C_4 53μ.
FIG. 6. C_1 $72\times74\times78\mu$.
FIG. 7. C_2 39μ.
FIG. 8. C_3 53μ.
FIG. 9. D_8 $37\times37\times40\mu$.
FIG. 10. E_9 $35\times35\times36\mu$.
FIG. 11. D_9 $27\times30\mu$.
FIG. 12. E_{14} $52\times54\times55\mu$.
FIG. 13. E_{16} 31μ.
FIG. 14. E_{15} 18μ.
FIG. 15. E_{10} $57\times58.5\mu$.
FIG. 16. E_{11} 52μ.
FIG. 17. E_{13} $59\times61\times65\mu$.
FIG. 18. F_3 $31\times48\mu$.
FIG. 19. F_5 $32\times40\mu$.
FIG. 20. G_1' $33\times49\mu$.
FIG. 21. G_2' $26\times35\mu$.
FIG. 22. G_3' 18μ.
FIG. 23. J_1 $53\times61\mu$.
FIG. 24. G_1'' $59\times78\mu$.
FIG. 25. H_1' $22\times26\mu$.
FIG. 26. J_3 Body diameter $44\times52\mu$.
FIG. 27. G_2'' 85μ.
FIG. 28. K_1 $58\times67\mu$.
FIG. 29. J_2 98μ.
FIG. 30. H_2' Average diameter $24\times30\mu$.
FIG. 31. J_4 Body diameter $59\times85\mu$.
FIG. 32. C_2 $35\times38\mu$.
FIG. 33. B_{13} $35\times48\mu$.
FIG. 34. E_{18} $27\times35\mu$.
FIG. 35. C_6 $45\times79\mu$.

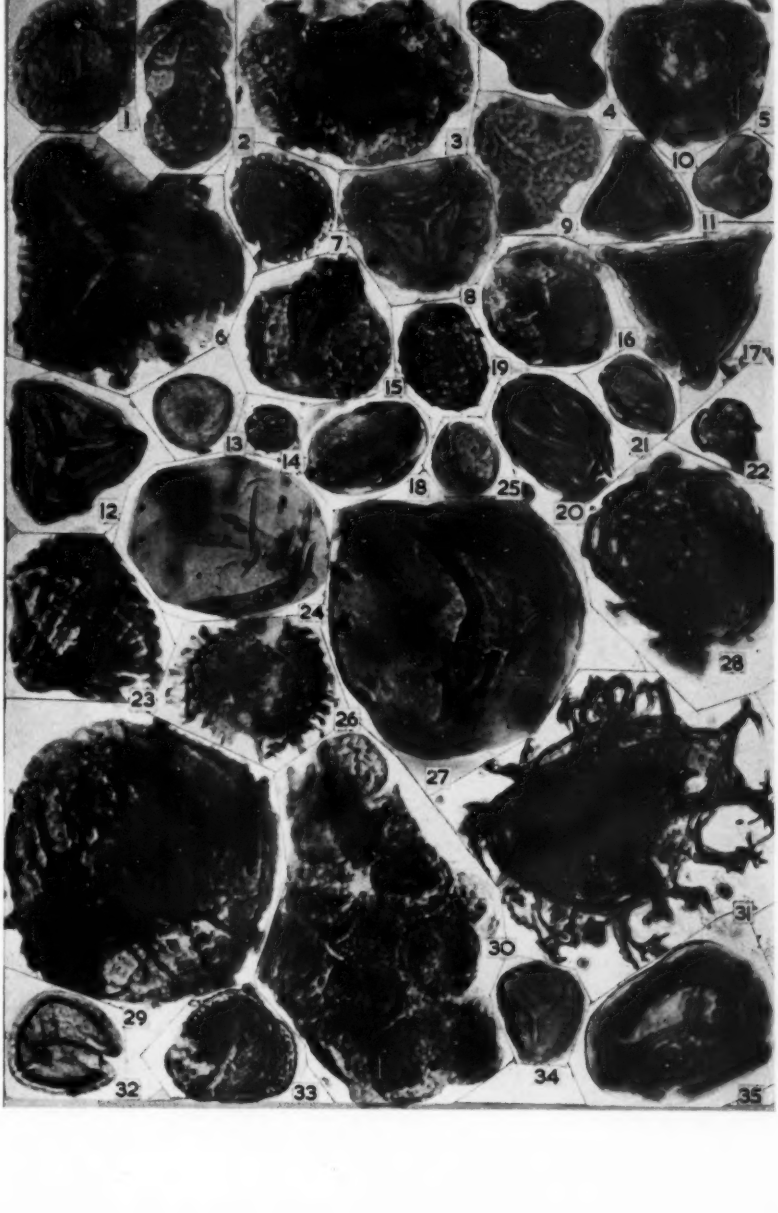
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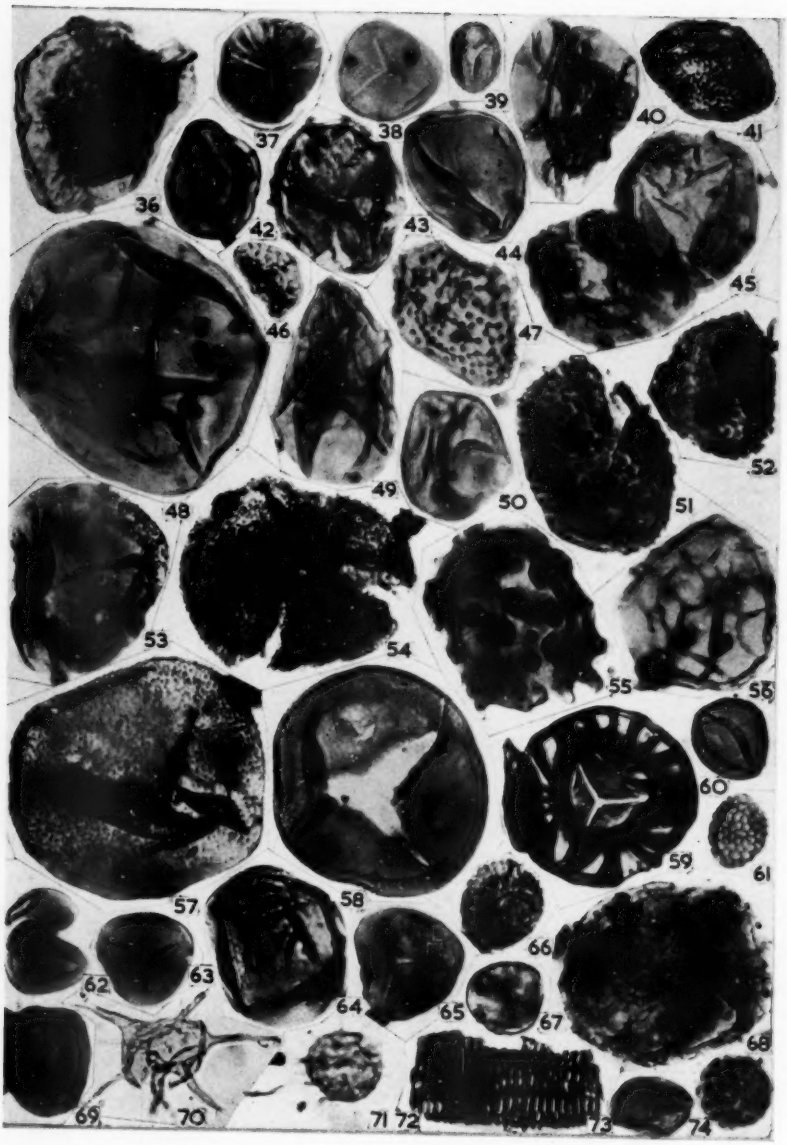
PLATE II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On one specimen (Fig. 2) there occurred a mark, parallel to the long axis of the grain, which may represent a monolete suture. Observed range of total spore diameter 60μ to 66μ; body diameter 39μ to 43μ. Body and air-sacs coarsely granular, the latter structures having in addition imperfect reticulations in one specimen.

In general form this type is similar to pollen of the modern genus *Pinus*. As is the case with several of the group B microfossils, type B_7 is referable to the genus *Pityosporites* Seward (30).

B_8—(*Fig. 3*)

Body outline circular to oval. Air-sacs not as broad as body, although because of distortion due to compression they may appear so in some specimens. Bladders tilted toward ventral (?) side of body, and narrowing slightly at point of juncture with body. No haptotypic features observed. Diameter range 62μ to 87μ between bladder extremities; body 52μ to 61μ in longest dimension. Ornamentation granulose.

This type was the most abundant group *B* microfossil in this horizon. It is referable to *Pityosporites*.

B_{12}—(*Fig. 4*)

Body outline oval to subrectangular. Air-sacs lateral, becoming broad at position of attachment to body. Wall folded in central region of body. Air-sacs each marked by two parallel lines or ridges situated 6μ to 7μ from lateral body margins. Haptotypic features not evident. Body diameter 22μ × 35μ; width between bladder apices 52.5μ; bladder width 17μ. Two specimens encountered of equal size.

This microfossil displays close resemblance to type N_2 of Radforth and Rouse (22). In the latter paper an affinity of type N_2 with *Pinus* is suggested.

C_1—(*Fig. 6*)

Outline subtriangular; margins of body between radii of spore concave; apices broadly rounded. Trilete rays distinct, extending to within 6μ to 8μ of the apices of the spore; commissure 4μ to 5μ wide near point of union of rays. Dimensions of spore including flange 72μ × 74μ × 78μ; at its greatest width the flange is 18μ wide. Margin of flange setaceous, greatest

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FIG. 36. C_5 58×75μ.
FIG. 37. E_{17} 42μ.
FIG. 38. E_{21} 39μ.
FIG. 39. E_{22} 18×26.5μ.
FIG. 40. E_{24} Diameter of tetrad 44×61μ.
FIG. 41. E_{27} 40×54μ.
FIG. 42. E_{28} 39×45μ.
FIG. 43. G_4' 56μ.
FIG. 44. G_5' 35×51μ.
FIG. 45. G_6' Diameter of group 53×90μ.
FIG. 46. H_3' 23×28μ.
FIG. 47. H_4' 41×61μ.
FIG. 48. G_3'' 96μ.
FIG. 49. G_1'' 44×70μ.
FIG. 50. H_3' 45μ.
FIG. 51. H_6' 50×68μ.
FIG. 52. H_7' 56μ.
FIG. 53. H_1'' 78μ.
FIG. 54. H_2'' 78×90μ.
FIG. 55. J_5 62×79μ.
FIG. 56. K_2 57μ.
FIGS. 57 to 66. Lower Devonian spores (Abitibi River, Ont.).
FIGS. 67 to 71. Silurian spores (Forillon Pen., Que.).
FIG. 72. Scalariform tracheids. Silurian (?) age (Charlotte County, N.B.).
FIGS. 73, 74. Silurian (?) spores (Charlotte County, N.B.).

length of setae being 10μ. In general, the setae are disposed as in *Reinschospora magnifica* Kosanke (12), but the condition of the setae in the present example makes accurate measurement difficult. Ornamentation granulose.

There is a high degree of resemblance of this specimen to *R. magnifica*, a spore isolated by Kosanke (12) from the Shoal Creek coal of Pennsylvanian age in Illinois. Affinity unknown. This type is classifiable as Pant's subgroup *Diatomozonosporites* (20).

C_2—(*Fig. 7*)

Outline roundly subtriangular. At the margin of the spore body is a dark band, which may indicate that the flange is slightly proximal or distal in attachment. Trilete rays extend into flange. Diameter range, including flange, 37μ to 39μ; flange 5μ to 6μ wide. Ornamentation granulose.

This spore bears resemblance to *Lycospora confinis*, figured by Cross (4), and on this basis lycopodaceous affinity is suggested. This type is referable to *Stenozonosporites* Pant (20).

C_3—(*Fig. 8*)

Outline subtriangular; margins of body between radii slightly concave; apices broadly rounded. Trilete rays distinct and open, extending to margin of spore body but not into flange. Flange thin, radially folded at corners of spore body. Total spore dimensions 46μ × 53μ; flange width 9μ to 12μ. Flange and body granulose.

This type is referable to *Hymenozonosporites* Pant (20). Not previously described.

C_4—(*Fig. 5*)

Outline roundly deltoid. A small trilete scar probably present on central body of figured specimen. Observed range of diameter including flange 53μ to 74μ; flange at greatest width constitutes 55% to 59% of total microfossil diameter. Body and flange finely obervermiculate; body less dense than flange.

This specimen shows some resemblance to *Hymenozonotriletes triangularis* described by Mehta (17). The major difference between Mehta's spore and the present example seems to be the larger size of the former spore (longest axis 124.7μ). This type may be placed in the genus *Euryzonosporites* Pant (20).

D_8—(*Fig. 9*)

Spore subtriangular; strongly concave at margins; apices broadly rounded. Trilete mark extends more than three quarters the distance to apices; commissure distinct. Spore diameter 40μ. Ornamentation verrucose.

This spore resembles Raistrick's type D_8 of lower Carboniferous age (23) in ornamentation and general form. However, Raistrick's spore has longer trilete rays and less concavity between body apices. Both of these are assignable to the subgroup *Verrucosisporites* Ibr. (20).

D_9—(*Fig. 11*)

Outline subtriangular; margins between radii broadly rounded. Trilete rays prominent, extending about half the distance to the spore margin. Spore dimensions $27\mu \times 30\mu$. Ornamentation finely granulose.

This type resembles Raistrick's D_4 (23). Kosanke (12) refers spores of this form to the Filicales (cf. *Granulati-sporites* (Ibr.) S.W. & B. (30)).

E_9—(*Fig. 10*)

Outline subtriangular; apices bluntly pointed. Wall slightly folded in angles of trilete suture. Trilete rays extend to within 4μ or 5μ of spore apices. Dimensions $35\mu \times 40\mu$. Wall levigate.

This type is referable to the subgroup *Laevigatisporites* Ibr. (20). Not previously described.

E_{10}—(*Fig. 15*)

Outline suboval, with a lobate fold extending to one side. Trilete mark distinct; rays *ca.* 27μ long. Spore dimensions $57\mu \times 58.5\mu$. Ornamentation of short blunt tubercles, 3μ to 5μ in length, tapering from 6μ or 7μ at their bases.

The manner of folding suggests that this spore may have originally been subtriangular in transverse plane. In size and ornamentation it resembles *Raistrickia prisca* Kosanke (12), which genus is closely related to the filicinean genus *Senftenbergia*. Type E_{10} may be placed in the subgroup *Tuberculati-sporites* Ibr. (20).

E_{11}—(*Fig. 16*)

Outline oval to slightly obovate. Trilete rays extend about half the distance to spore margin. Diameter 52μ to 63μ. Ornamentation granulose.

This type may be placed in the subgroup *Granulatisporites* Ibr. (20). Not previously described.

E_{13}—(*Fig. 17*)

Spore subtriangular in outline, with a distinct rounded prominence at each apex. Trilete mark present but partially obscured by coarse concentric striations of the wall. Dimensions of figured specimen $59\mu \times 61\mu \times 65\mu$, apical projections 9μ in length.

The ornamentation resembles that of Thiergart's *Mohria*-type (33, Plate III, Figs. 39, 40). Type E_{15} also shows some similarity to spores of *Anemia* (2, Fig. 451 L). Type E_{15} may be placed in the subgroup *Chomosporites* Pant (20).

E_{14}—(*Fig. 12*)

Outline subtriangular. Trilete rays extend two thirds of the distance to spore apices. Dimensions of figured specimen $52\mu \times 54\mu \times 55\mu$. Ornamentation of coarse, rather irregular ridges.

This type shows some resemblance to a microfossil isolated from oil-bearing Tertiary strata of Assam, India (28, Plate XVI, Fig. 37).

E_{15}—(*Fig. 14*)

Outline broadly oval; trilete scar prominent; spore diameter 16μ × 18μ; ornamentation papillate.

This type is referable to *Apiculatisporites* (B. & K.) emend. Ibr. (20). Not previously described.

E_{16}—(*Fig. 13*)

Outline roundly subtriangular. Trilete rays distinguishable, but not conspicuous. Evidence of contact areas between trilete rays. Diameter 31μ. Ornamentation finely granulose.

Spores resembling E_{14} have been described from Cretaceous and Tertiary coals (22, 33, 37) as species of *Sphagnum*.

F_3—(*Fig. 18*)

Outline elongate-oval. Monolete mark distinct. Diameter range 39μ to 48μ in greatest dimension. Spore wall usually slightly folded. Ornamentation granulose.

This type may be included in the subgroup *Azonomonoletes* Lub. (20). Microfossils of similar structure to group *F* have been described from Pennsylvanian (12), Upper Cretaceous (22), and Tertiary rocks (37).

F_5—(*Fig. 19*)

Outline oval to broadly bean-like. Range of dimensions 37μ to 40μ. Wall verrucose, obervermiculate.

This spore is similar to *Laevigato-sporites pseudothiessenii* Kosanke (12), a Pennsylvanian species. According to Kosanke (12, p. 28), "Suggested affinities (of *Laevigato-sporites*) based on modern plants strongly indicate a relationship with the pteridophytes". Schopf *et al.* (30, p. 36) mention the fact that "Smaller species of *Laevigato-sporites* agree with some forms obtained from filicinean type fructifications". Type F_5 may be placed in Pant's subgroup *Azonomonoletes* (20).

G_1'—(*Fig. 20*)

Spores elongate-oval or irregularly folded. Wall thin and levigate. Range of dimensions 46μ to 55μ.

G_2'—(*Fig. 21*)

Spore oval, slightly folded, and levigate. Diameter range 28.5μ to 35μ.

G_3'—(*Fig. 22*)

Microfossil circular and appearing trilobate in plane of compression. Widths of lobes 11μ, 12.5μ, and 12.5μ. Greatest microfossil diameter 18μ. Ornamentation finely granulose.

This microfossil apparently has the tricolpate condition, which is a common feature of some Mesozoic and recent angiosperm pollen grains. Its occurrence here is unique, since angiosperm pollens have not been previously reported

from strata older than Jurassic (32). The Jurassic occurrence was a grain of similar size and shape to G_3', both of these resembling grains of the Nymphaeaceous genus *Nelumbo*. In the present example, as in the Jurassic spore, ". . . . the furrows do not divide the grain into three equal parts. . . ." (32, p. 673).

G_1''—(*Fig. 24*)

Spore round to elliptical, thin-walled, transparent, and slightly folded. Observed diameter range 70μ to 79μ. Ornamentation levigate.

Except for the inevidence of a trilete scar, type G_1'' is identifiable with the genus *Calamospora* S.W. & B. (30) and is similar to some of the calamarian spores reported by Hartung (8).

G_2''—(*Fig. 27*)

Spore subcircular, thin-walled, and slightly folded, 84μ × 85μ in diameter. Wall granulose. Not previously described.

H_1'—(*Fig. 25*)

Outline broadly oval. Tetrad scar, if present, obscured by longitudinal folding of wall. Diameter 22μ × 26μ in each of two specimens recorded. Wall finely papillate.

H_2'—(*Fig. 30*)

Spores broadly oval to circular, 24μ to 32μ in diameter. Ornamentation verrucose.

These spores were arranged in a closely packed group of approximately 24 intact individuals. Associated with this group was a small amount of disorganized material similar in thickness and ornamentation to the spore walls. Not previously described.

J_1—(*Fig. 23*)

Spore subtriangular in plane of compression. Diameter 53μ × 61μ. There is present a well-defined oval area which is less dense than the remainder of the microfossil. Ornamentation of prominent, wide, flat-topped, parallel striations, apparently raised 2μ to 3μ above the spore surface. Width of striations 5μ to 6μ.

In size and ornamentation, this spore resembles some recent and fossil members of the Schizaeceae, in particular the genus *Mohria*, which has been described from the Cretaceous by Thiergart (33) and mentioned from Jurassic strata by Seward (31, p. 350).

J_2—(*Fig. 29*)

Spores oval, thin-walled, and slightly folded. Two specimens seen, of dimensions 87μ × 98μ and 96μ × 98μ. Wall granulose, with further ornamentation of thin, dark, subparallel ridges, from 7μ to 12μ apart.

This type compares well with Raistrick's Lower Carboniferous type G_1 (23).

J_3—(*Fig. 26*)

Spore circular, slightly broken at two points on margin. Diameter, not including setae, 44μ × 52μ. Ornamentation of closely spaced setae, 9μ in length and *ca.* 1μ in width. Some setae bifurcate at the tips. Setae apparent over most of the spore surface, although not always well preserved. Not previously described.

J_4—(*Fig. 31*)

Spore body irregularly oval. Diameter 59μ × 85μ exclusive of processes. Wall slightly folded, granular, bearing tapering bifurcate processes. Processes 16μ to 21μ in length, 8μ to 10μ wide at their base, and tapering to 3μ to 4μ before they divide.

Baschnagel (1) reports zygospores of Desmidaceae, from the Middle Devonian Onondaga limestone, which resemble J_4. Several of the Devonian spores described by Krausel (13, 14) also are similar to the present type, as is the postglacial genus *Hystrix* (6) which is believed to be a demid zygospore.

K_1—(*Fig. 28*)

Spore suboval, 58μ × 67μ in diameter. Wall ornamented by reticulations 6μ to 8μ wide with lamellae *ca.* 2μ wide. Margin shallowly crenate. Not previously described.

Spore Descriptions, Anse-à-Brillant Sample

B_{13}—(*Fig. 33*)

Detailed structure not clear, because of imperfect preservation. Air-sacs short but with broad locus of attachment to body. Body outline oval. Air-sacs meet body so as to make an angle of *ca.* 150° with one another. Body margin in this angle concave, bounded by slight lips or ridges near point of juncture with air-sacs. Opposite margin bulges outward. Haptotypic features not evident. Range of longest diameter 25μ to 52μ. Wall verrucose.

On the basis of its two tilted bladder-like appendages, this type may be a primitive gymnospermous pollen or prepollen related to the *Pityosporites* group. Not previously described.

C_2—(*Fig. 32*)

Cf. type C_2 of Wabamun Lake sample. Diameter 38μ.

C_5—(*Fig. 36*)

Outline subtriangular. Trilete mark present but poorly defined. Dimensions including flange 58μ × 75μ and 80μ × 113μ; dimensions of spore body 39μ × 50μ and 48μ × 85μ. Wall of flange slightly radially convoluted, semitransparent, and levigate; wall of body dense and vermiculate.

This type is referable to *Hymenozonosporites* in Pant's classification (20).

C_6—(*Fig. 35*)

Outline oblong to roundly subtriangular. Trilete rays extend to periphery of spore body but not into flange. Wall thickened in region of trilete mark,

and also thickened in a band surrounding body in vicinity of juncture with flange. Diameter range, including flange, 48μ to 79μ. Flange width 8μ to 13μ. Wall levigate.

Not previously described.

E_{17}—(*Fig. 37*)

Spore oval to subtriangular. Trilete rays distinct, extending to within 5μ of circumference of spore. Spore 38μ to 45μ in longest diameter. Wall levigate, opaque, and folded with radial folds extending from juncture of trilete rays to spore periphery.

This type is referable to Pant's subgroup *Laevigatisporites* (20). Not previously described.

E_{18}—(*Fig. 34*)

Outline subtriangular. Trilete commissure *ca.* 2μ wide, extending two thirds the distance toward the spore margin. Diameters of two examples: 27μ × 35μ and 28μ × 35μ. Wall papillate.

This type is referable to Pant's subgroup *Apiculatisporites* (20). Not previously described.

E_{21}—(*Fig. 38*)

Spore roundly subtriangular. Trilete aperture distinct, 2μ to 4μ in width, extending over three quarters the distance to the spore margin. Spore diameter 37μ × 39μ. Wall levigate.

This type is referable to Pant's subgroup *Laevigatisporites* (20). Not previously described.

E_{22}—(*Fig. 39*)

Oval in outline. Trilete aperture wide, and extending over half the distance to the spore margin. Spore diameter 18μ × 26.5μ. Wall finely papillate and slightly folded.

This type is referable to *Apiculatisporites* of Pant's classification (20). Not previously described.

E_{24}—(*Fig. 40*)

Tetrad of spores, tetrahedral in form. Diameter of tetrad 44μ × 61μ. Component spores, of approximately equal size, 24μ × 35μ. Walls folded, transparent, and levigate.

These spores are probably immature, since they have remained associated in the tetrad. At this stage they are classifiable as *Laevigatisporites* (20).

E_{27}—(*Fig. 41*)

Outline round to irregularly oval, usually folded. Trilete rays extend about half the distance to spore margin; rays obscured in many specimens by folding of wall. Diameter range 40μ × 50μ. Wall tuberculate, appearing finely reticulate when not in focus.

This spore is similar in size and ornamentation to *Lophotriletes impalbabilis* Naumova (19) which was described from a lower Cambrian blue clay near Timan. Type E_{27} is referable to *Tuberculatisporites* of Pant's classification (20).

E_{28}—(*Fig. 42*)

Spore irregularly elliptical in outline. Trilete rays 20μ long. Spore diameter 39μ × 45μ. Wall slightly folded, levigate.

This type is referable to *Laevigatisporites* (20).

G_4'—(*Fig. 43*)

Outline oval to subelliptical. Dimensions 39μ to 53μ. Wall finely granulose, dense, and usually folded.

G_5'—(*Fig. 44*)

Outline subelliptical to oval, subtriangular, or irregular. Dimensions 52.5μ to 61μ. Wall thin, levigate, semitransparent, and usually slightly folded.

Spores of this type are relatively numerous in the Anse-à-Brillant material, but they tend to be imperfectly preserved.

G_6'—(*Fig. 45*)

Spores usually encountered in groups of two. Outline subcircular, roundly subtriangular, or irregular folds. A gaping triradiate opening was evident in the figured example, but the point of union of the spore pair was not associated with this mark. Spore dimensions 49μ to 67μ. Wall finely granulose. Not previously described.

G_1''—(*Fig. 49*)

Cf. type G_1'' of Wabamun Lake sample.

G_3''—(*Fig. 48*)

Spores round to irregular, folded. Diameter in greatest dimension 70μ to 96μ. Wall finely granulose.

H_3'—(*Fig. 50*)

Spores round to subelliptical. Diameter in greatest dimension 29μ to 44μ. Wall finely papillate and often slightly folded. Not previously described.

H_4'—(*Fig. 47*)

Outline angular and irregular. Diameter 41μ × 61μ. Ornamentation of blunt processes up to 4μ in length, spaced 3μ to 6μ apart. Some of the processes jointed, and apparently arranged in short irregular rows. Wall between processes levigate. Not previously described.

H_5'—(*Fig. 46*)

Outline oval to irregular. Diameters 17.5μ × 25μ and 23μ × 28μ. Wall papillate-verrucose; projections 2μ to 3μ apart. Not previously described.

H_6'—(*Fig. 51*)

Outline elongate oval. Diameter 50μ × 68μ. Wall coarsely verrucose; projections 1.5μ to 2μ long, 3μ to 5μ apart. Not previously described.

H_7'—(*Fig. 52*)

Outline round to subtriangular. Diameter 57μ to 62μ. Wall coarsely verrucose; projections flat-topped, angular in plan view, 2.5μ to 4μ in diameter, 2.5μ to 3μ in length. In localized areas there is a suggestion of linear arrangement of the projections. In some instances the bases of the projections appear to coalesce to form shallow connecting ridges.

Type H_7' is comparable in size and ornamentation to some members of the genus *Raistrickia* S.W. & B. (30).

H_1''—(*Fig. 53*)

Outline irregularly oval. Range of dimensions 75μ to 91μ. Wall folded, and levigate except for certain areas, peripheral in plane of compression, which possess closely spaced short papillae. Not previously described.

H_2''—(*Fig. 54*)

Outline round to irregularly elliptical. Diameter 63μ to 100μ. Wall shagreen. Spores often broken or folded.

This type resembles *Trachytriletes krystofovichii* Naum. (19) except for the apparent lack of a trilete mark in the former spore.

J_5—(*Fig. 55*)

Outline subelliptical. Diameter 70μ to 79μ. Ornamentation of large, blunt processes, 5μ to 10μ wide and 6μ to 8μ long. Wall between processes granulose.

In form and pattern, this spore resembles *Senftenbergia pennaeformis* identified from an upper Carboniferous coal (21), with the exception that the connecting ridges of *S. pennaeformis* are not apparent in the present spore.

K_2—(*Fig. 56*)

Outline roundly subtriangular. Diameter 57μ to 70μ. Wall reticulate; reticulae 12μ to 13μ wide; lamellae 1μ wide; area between lamellae levigate. Spore margin shallowly crenate.

This type is similar in size and general features of ornamentation to some recent lycopod spores (9) and to some spores of the genus *Reticulatisporites* (cf. 11, Fig. 256).

Analysis of Results, and Conclusions

On the basis of the present evidence the land flora of the Devonian period was extensive and varied. This is contrary to the common interpretation. Also it is significant that conditions for deposition and preservation involved in at least two types of Devonian continental deposits are adequate for the preservation of spores and pollens. These circumstances, together with the

procurement and use—as demonstrated—of adequate extraction techniques, ensure availability of a wide range of material for study in a field comparatively new to research.

Specifically, the results imply the existence of a basis for dating and differentiating pre-Carboniferous continental deposits, using plant spores and pollens. Analysis of two strata indicates that a regional correlation may be possible by the use of the numerous morphologically distinct microfossil types peculiar to certain horizons. But since the degree of repetition within individual spore categories is low, and the diversity of types is high, only a portion of the total assemblage has good chance of repetition in the analysis of controls. This being the case, it is suggested that each assemblage, rather than specific constituents of the assemblage, will best serve to distinguish horizons.

On the other hand, the Gaspé and Alberta strata apparently possess some characteristic types. In the Wabamun Lake Upper Devonian assemblage, approximate percentages consistently show that categories G_1' (Fig. 20) and G_2' (Fig. 21) are present as over three per cent of the total. Although none of the other types showed this abundance, groups *B* and *F* were prominent constituents in a series of four duplicate macerations.

In the Gaspé Middle Devonian rock a characteristic microfossil type, E_{17} (Fig. 37), consistently constitutes approximately six per cent of the total assemblage. Three other types, C_6 (Fig. 35), E_{27} (Fig. 47), and G_5' (Fig. 44), are each present with a frequency of over three per cent. This suggests that if a more extensive investigation of these strata were undertaken their use could be widely exploited. Present investigation suggests that other equally useful strata will emerge.

Variation in assemblage already having been noted, it now seems safe to predict that for pre-Carboniferous strata as yet unexplored, other assemblages exist and their applicability to interpretations of oil strata seems probable. A high microfossil content and a diversity of floral groups may prove to be characteristic of other rocks associated with oil. In support of these suggestions, a recent preliminary examination of a lower Devonian sandstone from the area southwest of James Bay (Sextant formation), another potential oil-bearing area, has revealed a large and diversified spore population (Figs. 57–66). Preliminary investigations have in addition demonstrated the existence of relatively complex microfossils in Silurian rocks containing *Monograptus* from the Gaspé peninsula (Figs. 67–71) and in other rocks of probable Silurian age from Charlotte County, N.B. (Figs. 72–74). Work continues on each of these locations.

The results also require analysis from the botanical standpoint. Until recently it has not been clear how early complexity in form and pattern of microfossils began in the geological time scale. This work has shown that complexity of pattern and form in microfossils came into existence in a marked degree long before it could have been assumed from any previous evidence.

Complex morphological traits are displayed in the Upper Devonian Wabamun Lake level by several individuals with flange-like structures, with

air-sacs, or with monolete sutures and apparent bilateral symmetry. Also of special note with respect to complexity of pattern in both samples is the occurrence in some specimens of papillate, verrucose, ridged, reticulate, and otherwise ornamented rather than smooth exospores. The complexity noted largely parallels that already recorded for younger horizons. Several of the Wabamun Lake spores resemble types reported by various workers from Carboniferous and Mesozoic rocks (i.e., B_7 (Fig. 2), B_8 (Fig. 3), B_{12} (Fig. 4), C_1 (Fig. 6), C_2 (Fig. 7), C_4 (Fig. 5), D_8 (Fig. 9), D_9 (Fig. 11), E_{10} (Fig. 15), E_{16} (Fig. 13), F_3 (Fig. 18), F_5 (Fig. 19), G_3' (Fig. 22), and J_2 (Fig. 29)).

As regards spore form in the Middle Devonian, the Anse-à-Brillant sample presents less complexity than did the western sample, although there is still considerable diversity in this respect.

Spore complexity is also a feature of floras of the Lower Devonian and Silurian periods, as indicated by the microfossils of Figs. 57 to 74. Prominence of complexity (pattern and form) is on the other hand less obvious than for the Middle and Upper Devonian rocks.

If evaluations are to be made, based on this complexity of form and pattern, the attributes concerned must show organized relationship. Whether this organization is interpreted through natural or artificial directives matters little for many purposes. The present systems of classification used in palaeopalynology are essentially artificial, but their usefulness is widely acknowledged. The Key (Appendix I, p. 619) is another example of this type.

On the other hand it is always to be hoped that classification systems developed for microfossils will ultimately assist in revealing natural relationships among plants of past ages. Then, if microfossils are to make their best contribution to our knowledge of ancient plants, some means will have to be derived to relate natural relationship in microfossils to that in macrofossils.

Perhaps, in the meantime, assistance may be anticipated if even a single natural complex of traits can be suggested as applying for early microfossil assemblages. The mechanism of classification can be applied later and the relationship with macrofossils can then be evaluated as the opportunity arises.

In assessing pressure of natural traits the authors have turned to autogenetic evidence in spore pattern development. For this the developmental series known for the fossil Schizaeaceae have been utilized (21). Here, a progressive specialization of pattern can be assessed from the early Carboniferous to the present and this is known to be related to specialization of other parts of the plants giving rise to the spores. In the early members of the family there is relative lack of specialization of spore pattern. This is accompanied by primitiveness in form in sporangial structure (21), Upper Carboniferous, Cretaceous, and present-day Schizaeaceous spores demonstrate, on the other hand, relatively well organized spore patterns, accompanied by correspondingly higher organization in sporangial construction (2).

It may be held that the sequences emphasized here may be closer to phylogenetic than ontogenetic. The latter becomes clearer when spore development in a single species is contemplated, such as in *Senftenbergia plumosa* (21). Very young spores are smooth and thin walled. Later a granular to verrucose

condition arises, to be followed by papillae on wall thickenings which appear as ridges. The papillae finally become spinose and these show a bi- or tripartite splitting at their extremities.

Other examples of ontogenetic development and series are referred to by Knox (10).

A search for such natural sequences placed alongside the geological time scale has yielded evidence worthy of some consideration. An analysis of patterns, among spores from rocks ranging from Silurian to the Upper Devonian, leaves no doubt that complexity in pattern and form has increased in the course of geological time. The stages exemplified in the historical record are somewhat parallel to the ontogenetic series. Empirically, a relative lack of specialization in form obtains for the older horizons. Apart from spores which are evidently zygospores (Fig. 70), the commonly occurring types in the lowest horizons exemplify simplicity (Figs. 67, 69, 73). It is reasonable to emphasize that this is again the case for the Lower Devonian (Figs. 60, 62, 63, 64, 65). In the Middle Devonian there is an increase in tendency toward the utilization of wall thickenings to produce a pattern in the form of ridges and spines (Figs. 47, 51, 52, 55). Here too the bladdered type of organization makes its appearance (Fig. 33). Thus complexity of pattern and form is more marked than for the lower levels.

In the Upper Devonian, complexity in pattern shows marked advance. Not only are wall thickenings in the form of ridges present, but the ridges are organized in various ways (Figs. 12, 15, 17, 23, 28). The spinose effect remains as an established character. More striking still is the advance in form. At least four form factors are worthy of mention. One is shown in Figs. 9, 10, 11. This form combines with the ridged pattern of specialization to give the types shown in Figs. 12 and 17. Another type of modification is exemplified in Figs. 18 and 19 and a third is indicated in Figs. 5, 6, 7, and 8. The fourth level of specialization is represented by the extensive occurrence of the bladdered type of microfossil (Figs. 1, 2, 3, and 4). Thus, in the empirical sense, from Silurian to Upper Devonian, it is evident that trends toward greater complexity in both pattern and form are established.

That the trend from simplicity of wall and form to the spinose, ridged, and ridged spinose conditions shows paralleling sequences like the ontogenetic series exemplified in the developing spore pattern of *Senftenbergia* may be purely coincidental. In any case there is no firm evidence that the trend in geological time is a strictly phylogenetic one, since it cannot be proved that a natural relationship exists among the members participating in the trend. On the other hand the suggestion is there that increasing complexity in spore morphology as a biological trend, though not a taxonomic one, somewhat paralleled geological time from the Silurian through the Devonian. Recognition of these apparent biological trends makes it easier to appraise a conspectus of spores and pollens and on the basis of relative complexity of morphology to superficially judge its position in the geological scale. Tables I and II show this graphically.

TABLE I

REPRESENTATION OF SPORE PATTERN CHARACTERS IN SILURIAN AND DEVONIAN ROCKS

Geological period		Spore pattern								
		Smooth	Granulose	Ridged	Reticulate	Verrucose and papillate	Spinose	Ridged spinose	With long processes	Complexly organized
Silurian		+	+		+	+				
Devonian	Lower	+	+		+	+				+
	Middle	+	+	+	+	+	+	+		
	Upper	+	+	+	+	+		+	+	

TABLE II

REPRESENTATION OF SPORE FORM CHARACTERS IN SILURIAN AND DEVONIAN ROCKS

Geological period		Spore form						
		Round	Elliptic and oval	Tetrahedral	Bilateral	Specialized		
						Bladders	Flange	
Silurian		+	+					
Devonian	Lower	+	+	+				
	Middle	+	+	+		+	+	
	Upper	+	+	+	+	+	+	

It must also be emphasized that the conclusions developed here are empirical. Special cases do arise which must be regarded as exceptions. An example of this is represented in Fig. 59, a spore from the Lower Devonian. This spore is indeed highly specialized and only two examples of it were encountered. This and other infrequent examples (Figs. 61, 63, 66) emphasize that advanced specialization had been achieved in some cases in the early stages of land plant establishment.

Some attempts have been made to assign many of the examples in these assemblages to their natural groups. In the Alberta example, type C_2 (Fig. 7) is probably a lycopsid spore; G_1'' (Fig. 24) and G_2'' (Fig. 27) may be sphenopsid types; spores of the A and B groups are suggestive of a well-defined cordaitean–coniferous flora, and spores of group F and several of the trilete types (i.e. E_{10} (Fig. 15), E_{15} (Fig. 14), E_{16} (Fig. 13)) resemble fern spores. Certainly there is evidence of an abundant pteropsid component.

The spore aggregation of the Gaspé sample contrasts with that of the previous stratum with regard to the possible affinity of specific spore types. With the exception of C_2 (Fig. 7), G_1'' (Fig. 24), and G_2'' (Fig. 27), none of the eastern forms are common to the western assemblage. In the Gaspé rock microfossils of probable coniferous alliance are rare, only one type (B_{13} (Fig. 33)) being encountered. Monolete spores which suggested a filicinean component in the western assemblage were not found here. Representing lower plant groups are types G_6' (Fig. 45), G_1'' (Fig. 24), and H_1'' (Fig. 53), which are possible sphenopsid spores, and C_2 (Fig. 32), C_6 (Fig. 35), and K_2 (Fig. 56), which are similar to some lycopsid types. Few examples resemble known filicinean or bryophytic spores, but probably many of the trilete types are affiliated with these groups. On the basis of this evidence a lycopsid–sphenopsid floral component is indicated, with an undetermined but probably high incidence of pteropsid and bryophyte types as well.

It will be appreciated that the discovery of an apparent angiosperm pollen grain (type G_3' (Fig. 22)) in rocks as early as the Devonian is a matter which the authors would naturally hold in question. The earliest appearance of angiospermic pollen is generally accepted as Jurassic (32). Realizing the significance of their observations, the authors in their desire to be cautious at first considered rejecting the matter, especially since as yet the opportunity to extend the search beyond examination of the usual control samples has not been feasible. On the basis that the specimen existed, however, the view was subsequently held that its presence must be reported as pertinent evidence.

In questioning this matter, much weight was given to the possibility of contamination. The sample from which the pollen was derived was moved directly from the core, and in the laboratory all precautions were applied to avoid contamination. The authors are satisfied that the core material examined is lithologically characteristic for the mudstone parting, a feature which is marked and accepted by the geologists supplying the material. Also, it is to be emphasized that because the sample came from a core, and not from bore cuttings which might be questioned because of occasional cave-ins, the possibility of contamination is relatively negligible.

It is too early to conjecture into the prediction of plant types which must have borne most of the earlier microfossils, which have been revealed from Lower Devonian and Silurian rocks. The presence of scalariform tracheids (Fig. 72) points to either lycopoid or pteropoid groups. While this kind of tracheid supports the idea of a degree of specialization much higher than that exemplified in the Psilophytales, it does not assist in specifying whether the pteropsid group held greater prominence. It may be speculated that the filicinean group, rising into such prominence in the Carboniferous, was, in fact, reasonably well established in the early Devonian and late Silurian.

Spore specialization exceeds in degree that which would apparently obtain from an inspection of the psilophytalean complex, or from other complexes of vascular plants occurring in the Silurian and early Devonian horizons. It is tempting to suggest that this being the case, the plant organs from which the spores arose might also be complex, certainly more complex than the present known macrofossils belonging to these horizons would indicate. The apparent early complexity reflected in the microfossil evidence presented lends support for the concept of polyphylogeny. Future work on the Silurian and early Devonian strata will probably contribute more significantly to this claim.

Acknowledgments

The authors are indebted to the National Research Council for financial assistance and continued cooperation in aid of this investigation. Thanks are also gratefully extended to Dr. E. Shaw of Imperial Oil Limited and to the Geological Survey of Canada, for making available material for analysis.

Appendix I

Key to Spore Types

I. Air-sacs Present
- (*a*) One air-sac, encircling the spore body in the equatorial region ..... *A*
- (*b*) Two air-sacs, bilaterally disposed ..... *B*

II. Air-sacs Absent
- (*a*) Spores with an equatorial flange-like structure ..... *C*
- (*b*) Spores without equatorial flange-like structure:
  1. Spores trilete; round to subtriangular in transverse plane:
     - (i) Margins between trilete rays concave ..... *D*
     - (ii) Margins between trilete rays convex ..... *E*
  2. Spores monolete; round to subelliptical in transverse plane ..... *F*
  3. Spores with haptotypic features not evident:
     - (i) Wall smooth, punctate, or with fine granules:
       - (α) Spores small (usually less than 70μ) ..... *G'*
       - (β) Spores large (usually more than 70μ) ..... *G''*
     - (ii) Wall papillate, shagreen, or verrucose:
       - (α) Spores small ..... *H'*
       - (β) Spores large ..... *H''*
     - (iii) Wall setaceous, or with elongate processes, or ridged ..... *J*
     - (iv) Wall reticulate ..... *K*

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THE OCCURRENCE OF *FUSARIUM* SPECIES IN CANADA

IV. TAXONOMY AND PREVALENCE OF *FUSARIUM* SPECIES IN THE SOIL OF CEREAL PLOTS[1]

By W. L. Gordon[2]

Abstract

A taxonomic study was made of 12,485 isolates of *Fusarium* that were obtained from a total of 1674 soil samples taken from two series of permanent cereal plots during the years 1936–1942. Isolates of *Fusarium* averaged approximately 16% of total fungus isolates. A total of 16 species, varieties, and forms of *Fusarium*, classified in eight sections of the genus, were isolated. These species, varieties, and forms are *F. dimerum* Penz., *F. merismoides* Cda., *F. poae* (Pk.) Wr., *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., *F. concolor* Rg., *F. semitectum* Berk. & Rav., *F. acuminatum* Ell. & Ev., *F. equiseti* (Cda.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., *F. sambucinum* var. *coeruleum* Wr., *F. sambucinum* f. 6 Wr., *F. oxysporum* Schlecht, em. Snyder & Hansen, *F. oxysporum* var. *redolens* (Wr.) Gordon, *F. solani* (Mart.) App. & Wr. em. Snyder & Hansen. The majority of the species isolated were encountered each year. *F. oxysporum* and *F. equiseti*, together, accounted for approximately 75% of the total *Fusarium* isolates. Average concentrations per gram of soil of these two species were approximately 4800 and 2300 'units', respectively. Average concentrations per gram of soil of each of the remaining species isolated were below 600 units. Except for *F. dimerum* and *F. merismoides*, the species, varieties, and forms that were obtained from soil have been isolated previously from the basal parts and seed of cereals in the Prairie Provinces.

Introduction

Results of previous investigations carried out in the Prairie Provinces, with respect to the fungi that may be isolated from diseased basal parts of cereals, have shown that species of *Fusarium* almost invariably constitute part of the fungus flora associated with common root rot (2, 10, 11, 12). It was not always clear whether those species of *Fusarium* that were isolated from cereals affected by common root rot had been introduced into the soil by way of infected seed or other means and survived only in close association with the roots and other basal parts, or were a persistent part of the normal fungus flora of cereal-producing soils. The results of an investigation, undertaken by Bisby *et al.* (1), of the fungi that could be isolated from Manitoba soils by the plate method showed that species of *Fusarium* may be obtained by this method directly from soil samples. Some of the species of *Fusarium* isolated by them from the soil had previously been encountered among isolates of fungi from diseased root and basal parts of cultivated plants.

The present study was undertaken to determine the identity and relative prevalence of the species of *Fusarium* that may be isolated by the dilution-plate method directly from samples of soil taken each year, for several consecutive years, from the same cereal plots. This study formed part of a co-operative investigation carried out by the Plant Pathology Laboratory,

[1] *Manuscript received May 28, 1954.*

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Science Service, and the Bacteriology Department, University of Manitoba, Winnipeg, Man., to determine the microflora of cereal-producing soils in relation to the common root-rot problem in Manitoba.

Materials and Methods

The samples of soil from which *Fusarium* isolates were obtained by the dilution-plate method during this study were taken from two series of permanent plots,[3] a cultural and a fertilizer series, that were laid down in 1936 in the experimental field of the Plant Pathology Laboratory. This experimental field was located on the farm of the University of Manitoba, Fort Garry, Man., and had grown cereals for a number of years. The cultural sequence of the experimental field prior to 1936 was fallow in 1935, cereals in 1934 and 1933, and fallow in 1932. The soil is a dark brown, heavy clay that becomes compact and sticky when wet (6).

The cultural and fertilizer series of permanent plots each consisted of 36 100th-acre plots. Each series of plots was sown to wheat the first year, that is in 1936. Thereafter, plots in the cultural series were subject to different rotations whereas those in the fertilizer series received different fertilizer amendments.

During each of the first four years of this investigation, that is from 1936 to 1939 inclusive, samples of soil were taken from the 36 plots in each series. From 1940 to 1942 inclusive, however, it was necessary to reduce the number of plots from which samples of soil were taken to 18 from each series because of the amount of time and labor that was involved in the plating of 36 samples per week.

From 1936 to 1939 inclusive, a sample of soil was taken fortnightly from each of the 36 plots in the cultural series. In alternate weeks a sample was taken from each of the 36 plots in the fertilizer series. The first samples were taken each year during the early part of June and the last in September. Each year a total of seven samples was taken from each plot in each of the two series. The same procedure was followed in taking the samples from 1940 to 1942 except that only 18 plots in each series were involved. The procedure adopted for the sampling of plots was varied only when it became necessary to do so on account of abnormally wet soil conditions. In 1938 it was possible to take a total of only six samples from each of the 36 plots in the fertilizer series and in 1941 a total of only five samples from each of the 18 plots in each of the two series.

The methods employed in the sampling of the plots and in the dilution plating of the samples of soil have been published in detail by James and Sutherland (6), who co-operated in this investigation on behalf of the Bacteriology Department, University of Manitoba. Their procedure is briefly as follows: A composite laboratory sample of soil was first made from six soil cores, 6 in. $\times$ $1\frac{1}{4}$ in., that were taken at random from each plot at each

[3] *These two series of permanent plots were designed by, and were under the direction of Dr. F. J. Greaney, formerly on the staff of the Plant Pathology Laboratory, Winnipeg.*

time of sampling. After a moisture test had been run, sufficient soil was taken from each of the composite laboratory samples to give 25 gm. on a dry basis. A dilution of 1:5000 was used for making counts on the fungi present in the soil. One milliliter of soil solution was delivered to each of four Petri dishes. Czapek's medium, acidified, was employed for the fungus dilution plates. Counts of the fungi were made after the dilution plates had been incubated for four days at a temperature of 25°–28° C. The number of colonies of fungi that appeared on the plates ranged from 5–50 per plate, with most of the plates yielding a count midway between these limits.

The colonies of *Fusarium* that appeared on the dilution plates, along with other fungi, were transferred to tubes of potato sucrose agar for future identification studies. Transfer of these wild type colonies of *Fusarium* took place on the fifth or sixth day after plating. Potato sucrose agar was employed as a culture medium throughout the taxonomic study.

For the purpose of this paper, which is to make known the identity of the *Fusarium* isolates and the relative prevalence of each species, variety, or form isolated during this study, the data employed were derived from the same 18 plots of each series each year from 1936 to 1942. In addition, the data derived from the cultural and fertilizer series of plots were combined. By this procedure, therefore, the results of this study are based on a total of 1674 composite laboratory samples of soil, 846 of which came from the cultural series of plots and 828 from the fertilizer series during the seven-year period. A total of 252 composite laboratory samples of soil were plated each year in 1936, 1937, 1939, 1940, and 1942; 234 samples in 1938; and 180 samples in 1941.

Proportion of the Fungus Flora That Was *Fusarium*

From the data presented in Table I, it will be observed that the number of isolates of *Fusarium* that were obtained from the soil each year during this study constituted an appreciable percentage of the total number of fungus

TABLE I

Fusarium ISOLATES AS A PERCENTAGE OF THE TOTAL FUNGUS ISOLATES OBTAINED FROM THE SOIL OF THE SAME CEREAL PLOTS EACH YEAR FROM 1936 TO 1942

Years	Total No. of fungus isolates	No. of *Fusarium* isolates	Per cent *Fusarium* isolates
1936	11,267	1334	11.84
1937	15,526	2508	16.15
1938	11,040	1881	17.03
1939	10,224	1449	14.17
1940	10,158	1788	17.60
1941	8,278	1825	22.05
1942	10,449	1700	16.27
1936–42	76,942	12,485	16.25

isolates. The proportion of *Fusarium* isolates to total fungus isolates varied, however, from year to year. In 1936, *Fusarium* isolates constituted approximately 12% of the total fungus isolates whereas in 1941 they made up approximately 22%. During the other years of this study, the number of *Fusarium* isolates ranged from approximately 14 to 17% of the total fungus isolates (Table I). It may be of interest to note that in 1941, when the total number of fungus isolates obtained was the least, the proportion of *Fusarium* isolates was greatest (Table I). The converse of this relation of *Fusarium* isolates to total fungus isolates did not hold in 1937, however, for in that year the total number of fungi isolated was the greatest.

Of the 76,942 colonies of fungi that were counted on the dilution plates during the seven-year period, isolates of *Fusarium* accounted for 12,485, or approximately 16%. It is evident from the data given in Table I that *Fusarium* was a persistent part of the fungus flora of the soil during the years that the cereal plots were sampled.

Classification of *Fusarium* Species from the Soil

The system followed in naming and classifying the 12,485 isolates of *Fusarium* that were obtained from the soil of the cereal plots is the one adopted previously (4, 5) in naming and classifying the isolates of *Fusarium* that were obtained from the seed of cereals and other cultivated plants.

During this study, a total of 16 species, varieties, and forms of *Fusarium*, belonging in eight sections of the genus, were isolated from the soil. The species, varieties, and forms that were isolated, and the sections of the genus in which they are classified, are as follows:

Section Eupionnotes Wr.
- *F. dimerum* Penz.
- *F. merismoides* Cda.

Section Sporotrichiella Wr.
- *F. poae* (Pk.) Wr.
- *F. sporotrichioides* Sherb.

Section Roseum Wr.
- *F. avenaceum* (Fr.) Sacc.

Section Arthrosporiella Sherb.
- *F. concolor* Rg.
- *F. semitectum* Berk. & Rav.

Section Gibbosum Wr.
- *F. acuminatum* Ell. & Ev.
- *F. equiseti* (Cda.) Sacc.

Section Discolor Wr.
- *F. culmorum* (W. G. Sm.) Sacc.
- *F. sambucinum* Fuckel
- *F. sambucinum* var. *coeruleum* Wr.
- *F. sambucinum* f. 6 Wr.

Section Elegans Wr.
- *F. oxysporum* Schlecht. em. Snyder & Hansen
- *F. oxysporum* var. *redolens* (Wr.) Gordon

Section Martiella Wr.
- *F. solani* (Mart.) App. & Wr. em. Snyder & Hansen

Relative Prevalence of the *Fusarium* Species Isolated

There was a wide difference each year in the relative prevalence of several of the species, varieties, and forms of *Fusarium* that were isolated from the soil of the cereal plots. Data relating to the prevalence of each species,

variety, and form that was found each year in the soil, expressed as a percentage of the total isolates of *Fusarium*, are presented in Table II. It will be evident that *F. oxysporum* was by far the most prevalent species isolated. Approximately 50% of the total isolates of *Fusarium* obtained each year proved to be this species. Its preponderance was chiefly due to the presence of a single variety, namely, *F. oxysporum* var. *redolens* (Wr.) Gordon. Isolates of this variety were recorded separately from those of *F. oxysporum* in 1936, 1937, and 1938 only. During each of these years, they accounted for approximately 70, 65, and 60%, respectively, of the total isolates of *F. oxysporum*. *F. equiseti* comprised approximately 25% of the total number of isolates of *Fusarium* that were encountered in the soil during the seven-year period; this species may therefore be considered to have been commonly soil-borne. On the other hand, certain species appeared to be relatively rare in the soil of the cereal plots. Some of these species, namely, *F. dimerum*, *F. sporotrichioides*,

TABLE II

PERCENTAGE OF THE TOTAL *Fusarium* ISOLATES OBTAINED EACH YEAR FROM 1936 TO 1942 THAT WAS ACCOUNTED FOR BY EACH SPECIES OF *Fusarium* ISOLATED

Section and species	Percentage of total *Fusarium* isolates							
	Years							
	1936	1937	1938	1939	1940	1941	1942	1936–1942
Eupionnotes								
F. dimerum	0.45	0.92	—	—	—	—	—	0.23
F. merismoides	0.22	1.83	5.85	8.76	5.37	9.10	12.12	6.04
Sporotrichiella								
F. poae	0.45	0.16	0.26	0.21	0.22	0.11	0.47	0.26
F. sporotrichioides	—	0.04	0.05	—	0.17	0.11	—	0.06
Roseum								
F. avenaceum	0.60	0.84	0.16	0.07	0.11	0.05	0.06	0.30
Arthrosporiella								
F. concolor	—	0.08	—	0.07	0.11	—	—	0.04
F. semitectum	—	0.24	—	0.07	0.11	—	—	0.07
Gibbosum								
F. acuminatum	6.75	4.03	4.09	3.66	3.86	5.20	4.88	4.55
F. equiseti	28.86	30.75	29.51	19.19	25.39	23.18	14.64	24.95
Discolor								
F. culmorum	3.67	2.51	2.34	1.17	1.01	0.60	0.23	1.65
F. sambucinum	2.10	2.11	2.44	2.48	1.68	1.26	1.71	1.96
F. sambucinum var. *coeruleum*	1.80	4.47	4.89	4.14	4.19	1.97	2.29	3.51
F. sambucinum f. 6	—	0.04	—	—	—	—	—	0.01
Elegans								
F. oxysporum	50.97	48.10	46.52	55.62	53.35	52.99	59.06	51.91
Martiella								
F. solani	5.02	3.91	3.88	4.55	4.42	5.42	4.53	4.48
Total *Fusarium* isolates	1334	2508	1881	1449	1788	1825	1700	12,485

F. concolor, *F. semitectum*, and *F. sambucinum* f. 6, were not found each year of the seven-year period and, in those years that they were isolated, they comprised only a fraction of one per cent of the total isolates. Two other species, *F. poae* and *F. avenaceum*, were isolated each year but consistently accounted for less than one per cent of the total isolates. Each of the remaining species present in the soil, namely, *F. merismoides*, *F. acuminatum*, *F. culmorum*, *F. sambucinum*, *F. sambucinum* var. *coeruleum*, and *F. solani*, was isolated each year of the seven-year period but usually accounted for a relatively small percentage of the total isolates (Table II).

As indicated by their percentage of the total isolates, most of the species of *Fusarium* were fairly consistent in occurrence in the soil each year of the seven-year period. Fluctuations did occur, however, in the percentage of the total isolates which they attained from year to year. With the exception of possibly two species, namely *F. merismoides* and *F. culmorum*, there was neither a consistent upward nor a consistent downward trend from 1936 to 1942 in the percentage of the total *Fusarium* isolates that each species attained. There was, however, a rather distinct upward trend in the prevalence of *F. merismoides*, although not entirely without some year-to-year fluctuations (Table II). This species accounted for only a fraction of one per cent of the total *Fusarium* isolates obtained in 1936, whereas in 1942 the isolates of this species attained approximately 12% of the total. In *F. culmorum*, the trend was the reverse of that in *F. merismoides*. This species accounted for approximately three and one-half per cent of the total isolates in 1936, but in each of the later years gradually accounted for a smaller percentage of the total, until, in 1942, it comprised less than one-quarter of one per cent. No explanation can be offered for this gradual decline of *F. culmorum* in the soil of the cereal plots.

The relative prevalence of the species, varieties, and forms of *Fusarium* that were isolated may also be compared with respect to their concentration per gram of soil. The term concentration is employed here to denote the number of viable 'units' of *Fusarium* that were present in each gram of soil. It is recognized that these viable units of *Fusarium* may be macroconidia, microconidia, chlamydospores, or hyphal cells, singly, or in small groups. The average concentration of each species, variety, and form per gram of soil in each year of the period, 1936 to 1942, was calculated by the formula:

$$\text{Average concentration per gram soil} = \frac{\text{dilution}}{\text{plots} \times \text{samples} \times \text{plates} \times \text{years}} \times \text{total isolates of each species, var., or form.}$$

The various species, varieties, and forms of *Fusarium* that were isolated may be classed into three groups according to their average concentration per gram of soil. The first group of species, with a relatively low average concentration per year, for the seven-year period, of less than 30 units per gram of soil, consists of *F. dimerum* (22 units), *F. poae* (24), *F. sporotrichioides* (5), *F. avenaceum* (28), *F. concolor* (4), *F. semitectum* (7), and *F. sambucinum* f. 6 (less than 1 unit). Species included in the second group, with average con-

centrations that ranged between 150–600 units approximately, are *F. merismoides* (563 units), *F. acuminatum* (424), *F. culmorum* (154), *F. sambucinum* (183), *F. sambucinum* var. *coeruleum* (327), and *F. solani* (417).

The third group, with a relatively high average concentration per gram of soil, consists of two species only, *F. equiseti* and *F. oxysporum*. The former species had an average concentration per gram of soil of 2324 units and the latter, 4834. These two species of *Fusarium* would appear to have been strongly entrenched in the soil of the cereal plots that were sampled.

Discussion

The results of this study have shown that an appreciable proportion of the fungus flora of the soil in the cereal plots, as determined by the dilution-plate method, consisted each year of *Fusarium*. The species, varieties, and forms that made up the *Fusarium* flora of the soil are of interest particularly because of their previously known association with cereals. With the exception of *F. dimerum* and *F. merismoides*, which in Manitoba have been found only in soil, the species, varieties, and forms that were isolated from the soil are known to be harbored by cereal seed (3, 4), and have been associated with common root rot of cereals in the Prairie Provinces (2, 12).

It is worthy of note that three species of *Fusarium* that are generally conceded to be of importance in cereal pathology were apparently absent in the soil of the cereal plots. These three species are *F. graminearum* Schwabe, *F. moniliforme* Sheld. em. Snyder & Hansen, and *F. nivale* (Fr.) Ces. Although the first two species are occasionally found on cereals in Manitoba, the third is not known to occur in this province.

The isolation of *F. culmorum* from the soil each year is of particular interest because of the relation of this species to common root rot of cereals in Manitoba. While the concentration of this species per gram of soil was relatively low in each of the seven years that this study was conducted, compared with that of *F. oxysporum* and *F. equiseti*, this species would appear to be part of the persistent *Fusarium* flora of the soil in plots that were sampled.

Probably the most striking result obtained from this study, with respect to the occurrence of *Fusarium* in the soil of the cereal plots, was the preponderant supremacy of *F. oxysporum* over the other species, varieties, and forms. Each year, this species accounted for approximately 50% of the *Fusarium* flora. The preponderance of this species was chiefly due to the presence of a single variety, namely *F. oxysporum* var. *redolens*. Although *F. oxysporum* is of recognized economic importance because of its ability to cause vascular wilts of many non-cereal crops by reason of its numerous pathogenic strains, this species is not recognized as an important pathogen of cereals in Canada.

As a result of their investigation on the *Fusarium* flora of the different types of soil found in Central America, Reinking (7), and Reinking and Manns (8, 9) concluded that species of this genus that were relatively abundant and widely distributed in the soil may be designated as soil inhabitants whereas those species that were less abundant and less widely distributed may be regarded

as soil invaders. Whether or not the species that were isolated from the soil of the cereal plots during the present study are to be regarded as soil inhabitants or as soil invaders depends upon the criteria employed to differentiate these two classes. If only those species that were isolated from the soil each year of the seven consecutive years that this study was continued and were present in a relatively high concentration per gram are to be regarded as soil inhabitants, then two of the species isolated, namely, *F. equiseti* and *F. oxysporum*, may be placed in this class. However, if continued presence in the soil each year of the seven-year period is considered to be the criterion of greatest significance and if concentration per gram of soil is of minor consequence in determining whether or not a species is a soil inhabitant or a soil invader, then *F. merismoides*, *F. poae*, *F. avenaceum*, *F. acuminatum*, *F. equiseti*, *F. culmorum*, *F. sambucinum*, *F. sambucinum* var. *coeruleum*, *F. solani*, as well as *F. equiseti* and *F. oxysporum* may be regarded as soil inhabitants, as these species were present in the soil each year. Of the species that were isolated from the soil, *F. dimerum*, *F. sporotrichioides*, *F. concolor*, *F. semitectum*, and *F. sambucinum* f. 6 do not qualify as soil inhabitants on the basis of the criteria considered, as they were not isolated each year.

Whether the *Fusarium* flora of the plots sampled during this study is typical of the soils of different type found elsewhere in Manitoba, or is only characteristic of the type of soil in the area where the plots were located, requires further investigation.

Acknowledgments

The writer is indebted to Dr. J. E. Machacek for the records of "total number of fungus isolates" and to Margaret E. Lathe who assisted in the preparation of the manuscript.

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STUDIES IN FOREST PATHOLOGY

XIV. DECAY OF DOUGLAS FIR IN THE COASTAL REGION OF BRITISH COLUMBIA[1]

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Abstract

An investigation of Douglas fir, *Pseudotsuga taxifolia* (Poir.) Britton, in coastal British Columbia has shown that decay losses in old-growth stands are low, amounting to 2.6 and 7.3% of the gross volume of living and combined living and dead trees respectively. Twenty-five decay-producing fungi were isolated from living trees and 29 from dead trees. The most important of these were *Fomes pini* (Thore) Lloyd, *Polyporus schweinitzii* Fries, and *Fomes pinicola* (Sw.) Cke. Certain irregularities in the occurrence of decay-producing fungi and the amounts of decay associated with them were traced to the influences of site, tree age or size, latitude, and stand history. Root infections were the most frequent but branch-stub infections caused the greatest average amount of decay. An examination of tree abnormalities showed that a select group of them, sporophores and swollen knots of *Fomes pini* in particular, are useful indicators of hidden decay. A separate analysis of dead trees has shown that they occasionally form a high proportion of Douglas fir stands and that much of the wood in such trees is sound.

Introduction

The dependence of the lumber industry of coastal British Columbia upon old-growth Douglas fir (*Pseudotsuga taxifolia* (Poir.) Britton) is traditional, the many excellent qualities of this species having established a preference for Douglas-fir lumber in both world and domestic markets. More than 40% of the volume of trees cut in coastal British Columbia in 1952 was Douglas fir, the 1952 cut of this species being 238 million cu. ft. (13). The volume of mature Douglas fir remaining in coastal forests was, as at December, 1952, 6868 million cu. ft. (13). In view of its sustained large annual cut and of the fact that Douglas fir of such quality cannot be regarded as a renewable resource, the practical liquidation of old-growth Douglas fir may be expected to occur within a few decades. Although its eventual liquidation seems inevitable, it is hoped that through careful management an abrupt decline in production from this unique timber resource may be avoided. Basic to the management of the remaining stands of mature Douglas fir is a reliable inventory of their gross and net volumes and an appreciation of the influences that operate for and against the maintenance of such stands. In response to requests of the lumber industry and of the provincial forest service, the Victoria Forest Biology (Pathology) Laboratory made an investigation of decay in stands of old-growth Douglas fir during the period 1947-52. This paper reports upon the findings of that work.

[1] *Manuscript received May 31, 1954.*
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Review of Related Investigations

Investigations of decay of mature Douglas fir have centered in the works of Boyce (1, 2, 3, 4), Kimmey (9), and Boyce and Wagg (5). The works of Boyce have particular application to western Oregon and Washington, while that of Kimmey concerns northern California, and that of Boyce and Wagg deals mainly with western Oregon. There are no known records of work done prior to this investigation that is based on British Columbia conditions. Consequently, the findings of Boyce, culminating in his 1932 report (4), have provided the only guide to an evaluation of decay of Douglas fir in British Columbia during the past 20 years.

In his final report (4), which was based on 2633 living Douglas firs on 38 plots, Boyce found that decay caused a loss of 9.5% of the total cubic volume of the trees examined and that red-ring rot, caused by *Fomes pini* (Thore) Lloyd, caused 79.2% of all the decay encountered. Most of the remaining decay was ascribed to brown trunk rot (*Fomes officinalis* (Vill. ex Fr.) Faull), red-brown butt rot (*Polyporus schweinitzii* Fries), and yellow-brown top rot (*Fomes roseus* (Alb. and Schw. ex Fr.) Cke.). With respect to the relation of tree age to decay, Boyce found that decay increment did not exceed gross increment until about 215 years under the very worst conditions on Site II land and not until 300 years under average conditions on Site II land. With respect to latitudinal differences in decay loss, he found that the percentage loss from decay was much higher in his southern plots than in those farther north, except in the younger age classes where no apparent difference occurred. With respect to the relation of site to decay, Boyce noted that, in the younger age classes at least, decay was of least consequence on good sites and was more serious on intermediate sites.

Kimmey investigated decay of Douglas fir in northwestern California from the aspect of cull factors that were based on the occurrence of external indicators of hidden decay (9). His approach to a consideration of decay of Douglas fir recognizes the fact that in all stands only some trees are decayed, many being entirely sound. His approach assumes that most of the decay is indicated by one or more of several types of decay indicators, the volume of decay that remains hidden being held to be of no consequence. He derived 16 cull-indicator classes that were designed to provide cull factors for individual trees according to the presence on them of decay indicators or combination of decay indicators. Using these 16 cull-indicator classes he presented the percentages of cull that may be expected in Douglas fir for a range of sites and tree sizes. His cull varied from 100%, for trees having either a single sporophore of *Fomes officinalis* or having *Fomes pini* sporophores that are separated vertically more than 10 ft., to smaller cull factors for trees that lack sporophores. In general, his cull factors were less for trees on good sites than for those on poor sites.

The work of Boyce and Wagg (5) substantiates the basic findings of Boyce's earlier works (1, 2, 3, 4) and, in addition, presents a thorough analysis of many

aspects of *Fomes pini* decay that had not been considered previously. In general, their investigation, based on the dissection of 292 Douglas firs on 14 plots representing highly-defective stands, re-affirmed the dominance of *F. pini* decay over all other decays. They considered *F. pini* decay from the viewpoints of its occurrence in stands and in individual trees.

In the case of decay in living trees, Boyce and Wagg found that both the base and the top of the decay column increased in height above ground with increasing tree age. They relate this fact to the dropping out from stands of the trees infected earlier and to the fact that the newer infections occur higher in older trees. They found also that *F. pini* infected fast-grown trees more than slow-grown trees. Further to their conclusion that fast-grown trees are highly subject to *F. pini* infection, they believe *F. pini* infection to be cyclic, the periods of low infection corresponding to periods of stand development during which the number of trees per unit area is being reduced through competition. They believe the periods of high infection to correspond to periods of stand development during which many trees have a high growth rate such as occurs when competition between trees is less intense. The authors state that, once infected, a tree becomes progressively decayed until the sapwood is encroached upon, whereafter a decline in growth rate and subsequent loss of dominance occurs. Such highly defective, slow-growing trees are believed to drop out of a stand, thus releasing from competition their less defective neighbors and thereby initiating a new cycle of infection. The authors believe that two or more cycles of *F. pini* infection occur in the life of a Douglas fir stand. They found that infection cycles were initiated earlier on good sites than on poor sites.

In addition to demonstrating a cyclic occurrence of *F. pini* infection Boyce and Wagg demonstrated a parallel cyclic change in percentage loss from decay. They believed the cyclic change in percentage loss to be the result of a reduction in the amount of decay in living trees, caused by the death and dropping out of highly-defective individuals, along with the subsequent increased growth of the comparatively sound survivors. Consequently, in the opinion of the authors, it may be necessary on occasion to postpone cutting old-growth stands that are currently highly defective in anticipation of greater yields at a later date.

The authors found that decay was much more consequential on southerly aspects than on northerly aspects and that steep slopes and upper slopes were more conducive to a high degree of infection. Soil was found to influence the degree of *F. pini* infection in some of its aspects, trees on well-drained, shallow, nitrogen-rich soils often being characterized by heavy infection. Soil texture and acidity bore no relation to the incidence of *F. pini*. The proportion of Douglas fir in a stand also appeared to influence the severity of *F. pini* infection, stands with low concentrations of Douglas fir being more free from *F. pini* than stands having high concentrations of Douglas fir.

Location of the Areas Investigated

The areas investigated were situated in the coast forest between latitudes 48° N. and 53° N. and had an altitudinal range of from 300 to 2300 ft. above mean sea level. Their approximate locations are indicated in Fig. 1. Nineteen of the 21 areas investigated were on Vancouver Island and two were on the mainland near the coast.

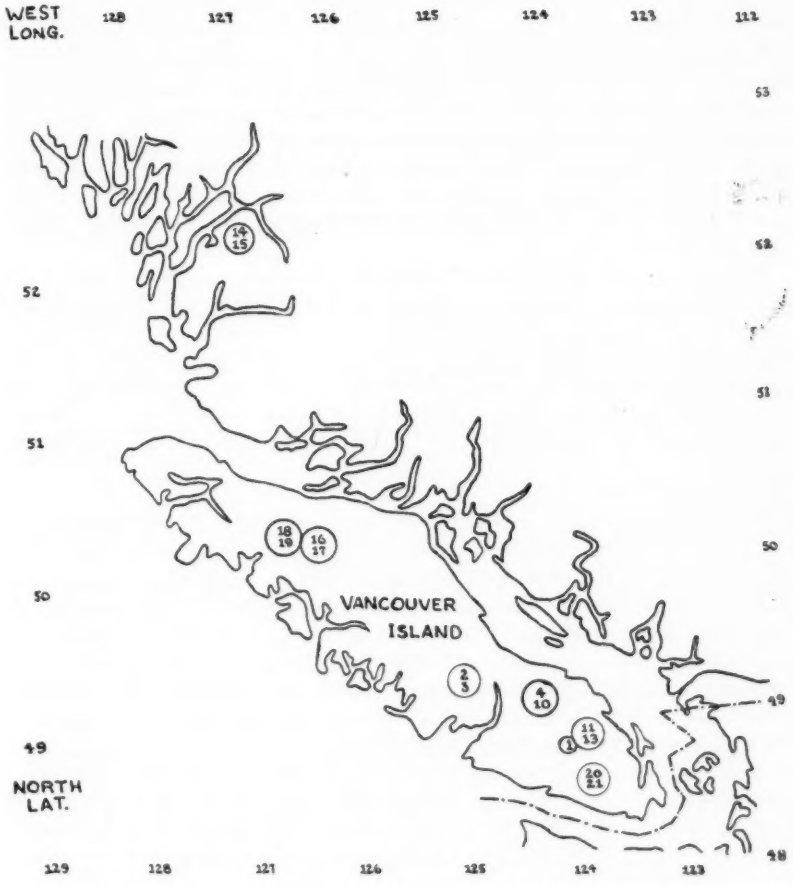

FIG. 1. Map of the areas investigated. The circles represent the approximate locations of the areas sampled, as follows: (1) Cowichan Lake, (2-3) Sproat Lake, (4-10) Nanoose, (11-13) Chemainus, (14-15) Bella Coola, (16-17) Woss Lake, (18-19) Nimpkish Lake, (20-21) San Juan.

Methods

Field procedures were generally in accordance with standard procedures of the Victoria Laboratory (8) and departures were made from these standard procedures only as required to meet the special demands of the investigation. Since all sampling was done in conjunction with the current logging of the co-operating companies, the sizes of the trees and logs available for analysis conformed to the standards of the companies concerned.

Before Felling

Temporary plots of from $\frac{1}{4}$ to $\frac{2}{5}$ ac. in size were established in standing timber, a number of plots comprising a sample area. The individual plots were approximately uniform as to site quality and tree age, while individual sample areas were usually uniform only as to site quality. The size of sample areas varied from $\frac{1}{4}$ to $4\frac{1}{2}$ ac. The site quality of the plots was evaluated according to the methods used by McArdle, Meyer, and Bruce (10). Thus, five Douglas fir site-quality classes were designated, with increasing growth capacity, as sites V to I.

Both living and dead trees of all species on the plots were tallied to a minimum diameter of 6 in., according to their diameter at breast height (d.b.h.)[4] and as being either understory[5] or merchantable[6]. In addition to their diameter, the dominance class[7], height[8], and the location of tree abnormalities were recorded for merchantable trees. The ages of understory trees and the characteristics of the slope, aspect, elevation, ground cover, and soil were also recorded for each plot. Only trees of merchantable size were felled.

After Felling

The felled trees were cut into lengths of from 16 to 40 ft. until a minimum top diameter of 10 in. was reached. The decayed portions of trees were measured as they were seen on stumps and log ends, no allowance being made for incipient decay unless such decay was clearly indicated by discolored wood. In cases of heartwood decay that showed at both ends of logs, the volumes of decayed wood were calculated according to the formula $\frac{1}{2}$ (basal area of decay showing at the base $\times$ basal area showing at the top) $\times$ log length.[9] In cases of heartwood decay that showed at only one end of logs, the volumes of decayed wood were calculated according to the formula $\frac{1}{2}$ basal area of decay $\times$ $\frac{1}{2}$ log length. In cases of sapwood decay the exact longitudinal and radial dimensions of the decayed wood were recorded and the volumes of wood so affected were thus calculated. The volumes of decayed wood in stumps and tops were not determined. The species of fungi that were associated

[4] *d.b.h. = the diameter in inches, inclusive of bark thickness, measured at 4½ ft. above the ground line.*

[5] *Trees 6 to 14 in. d.b.h.*

[6] *Trees 15 in. d.b.h. and larger.*

[7] *Four classes used: dominant; co-dominant; intermediate; overtopped.*

[8] *Heights were measured by Abney level.*

[9] *Basal areas calculated in sq. ft. Log lengths in ft.*

with decayed wood were determined, in most cases, on the basis of examinations of cultures obtained from plantings of infected wood upon malt agar slants and plates[10] and, to a lesser degree, on the basis of sporophore associations.[11]

The dimensions of trees and the decayed portions in them were recorded on Science Service Form SB 246 in the field. The volumes of trees and decayed wood were determined in the laboratory. The lengths of trees and logs were measured to the nearest whole foot. Diameters were measured to the nearest whole inch. The total height of a tree was obtained by summing its stump height, its log lengths, and the length to the top above the last log cut. Diameter measurements were made at stump height, breast height, at 16 ft. above ground level, at log ends, and at points of breakage. No measurements of tree diameter were made above a 10 in. top. Tree volumes were calculated in cubic measure, according to Smalian's formula, as the sum of log and piece volumes. Such volumes were termed gross-merchantable volumes, being the total volume between stump height and a 10 in. top, and served as a basis upon which to evaluate the significance of decay.

Douglas Fir Characteristics

Two forms of a single species of Douglas fir occur in British Columbia. This report refers to only the coast form, *Pseudotsuga taxifolia* (Poir.) Britton, which is confined to the islands and mainland coast. The second form, *P. taxifolia* (Poir.) Britton var. *glauca* (Mayr) Sudw., occurs east of the range of the coast form (6). The coast form grows north to Gardner Canal on the mainland but ceases to be a regular component of coastal forests north of Knight Inlet on the mainland and the Nimpkish Valley on Vancouver Island.

Douglas fir is characteristically a large tree at maturity and reaches advanced ages. It has an exceptionally long, branch-free stem at maturity, a thick bark, and a well-branched root system. Douglas-fir heartwood varies from being slow-grown and fine-grained to fast-grown and coarse-grained. It is moderately light in weight, stiff, high in impact resistance, and is moderately durable.

The origin of the forest soils that support coastal Douglas fir is largely glacial till, glacial outwash, or alluvial deposit. Most of such soils are podsolized, the best growth of fir being obtained in weakly-podsolized, beta- or gamma-gley soils. The humus of Douglas fir soils varies from duff mull to raw humus, with maximum tree growth being obtained in soils with duff-mull humus. Douglas fir tolerates a broad range of soil acidity but appears to reach its best development in soils having an average pH of 6.0–5.5.

[10] *The authors are indebted to Dr. M. K. Nobles, Senior Mycologist, Science Service, Department of Agriculture, Ottawa, and her associates for their careful examinations of all cultures submitted with respect to this investigation.*

[11] *The authors are indebted to Dr. J. W. Groves, Senior Mycologist, and Dr. R. Macrae, Forest Mycologist, Science Service, Department of Agriculture, Ottawa, for the naming of sporophores sent to them in the course of this investigation.*

Young Douglas fir stands tend to be even-aged and pure but, as stands become older, they become uneven-aged to the extent that several age groups may occur in a single stand. Likewise, stand purity is less common at maturity than at young ages, particularly on good sites. The main coniferous associates of coastal Douglas fir are western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western red cedar (*Thuja plicata* Donn), grand fir (*Abies grandis* (Dougl.) Lindl.), and western white pine (*Pinus monticola* Dougl.).

The Basic Sample

Twenty-one sample areas provided 2148 merchantable-sized Douglas firs with a gross merchantable volume of 638,453 cu. ft. for analysis. The same areas supported a living understory of 2243 trees of which 133 were Douglas fir, 1453 western hemlock, 644 western red cedar, 9 western white pine, and 4 grand fir. Each sample area consisted of from 1 to 18 temporary plots, a total of 141 plots being used within a site range of V to I and an average-age range of from 116 to 381 years. The basic data obtained from the 141 plots investigated are presented in Tables I and II.

TABLE I

THE LOCATION, EXTENT, AND NATURE OF 21 AREAS OF DOUGLAS FIR SAMPLED IN COASTAL BRITISH COLUMBIA

Area	Location	Site class	Plots		Number of living trees*	Percentage of the stand Douglas fir
			Number	Extent (ac.)		
1	Cowichan Lake	IV	5	1.25	289	73.0
2	Sproat Lake	III	10	4.00	376	60.6
3	Sproat Lake	II	5	2.00	207	57.4
4	Nanoose	V	1	0.25	20	60.0
5	Nanoose	IV	15	3.75	308	62.3
6	Nanoose	III	5	1.25	111	55.0
7	Nanoose	IV	3	0.75	55	49.0
8	Nanoose	III	13	3.25	242	69.4
9	Nanoose	V	1	0.25	21	38.1
10	Nanoose	IV	18	4.50	511	41.3
11	Chemainus	III	1	0.25	13	61.5
12	Chemainus	II	16	4.00	283	45.2
13	Chemainus	I	2	0.50	27	70.4
14	Bella Coola	III	5	1.25	100	52.0
15	Bella Coola	II	11	2.75	144	75.0
16	Woss Lake	II	12	3.00	159	45.9
17	Woss Lake	I	2	0.50	28	35.7
18	Nimpkish Lake	III	4	1.00	104	86.5
19	Nimpkish Lake	II	4	1.00	121	81.8
20	San Juan	III	5	1.25	111	56.8
21	San Juan	II	3	0.75	61	49.2
Total or average			141	37.50	3291	58.2

* *Trees of all species 15 in. d.b.h. and larger.*

TABLE II

BASIC DATA APPLICABLE TO A 37-ACRE SAMPLE OF DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Area	Living Douglas fir				Dead Douglas fir				Total Douglas fir			
	Number	Average age	Gross volume	Percentage of gross volume decayed	Number	Average age	Gross volume	Percentage of gross volume decayed	Number	Average age	Gross volume	Percentage of gross volume decayed
1	211	240	21,250	0.9	14	219	1022	74.7	225	239	22,272	4.3
2	228	240	42,751	1.4	5	234	856	37.9	233	240	43,607	2.1
3	119	373	97,887	2.0	10	375	5388	42.9	129	373	103,275	4.1
4	12	355	1217	7.4	1	294	48	81.3	13	350	1265	10.1
5	192	358	36,834	3.5	16	345	1283	92.0	208	357	38,117	6.5
6	61	355	18,362	3.3	2	336	378	100.0	63	354	18,740	5.2
7	27	314	4038	5.0	2	348	162	100.0	29	315	4200	8.6
8	168	275	42,936	1.1	68	268	13,786	54.9	236	273	56,722	14.2
9	8	288	203	2.9	1	283	20	85.0	9	287	223	10.3
10	211	286	19,766	0.4	19	295	1082	65.8	230	287	20,848	3.8
11	8	361	5557	1.2	2	336	795	26.5	10	356	6352	4.4
12	128	367	87,688	3.5	33	348	14,541	33.5	161	362	102,229	8.1
13	19	381	14,377	3.3	2	389	1387	67.6	21	382	15,764	8.1
14	52	268	20,476	11.7	12	259	2586	76.7	64	266	23,062	19.0
15	108	269	42,260	6.7	15	256	3210	70.0	123	267	45,470	10.0
16	73	381	58,502	0.5	19	354	9603	60.0	92	376	68,105	8.9
17	10	381	10,493	0.7	5	367	2516	59.6	15	376	13,009	12.1
18	90	118	6455	Trace	Nil	—	—	—	90	118	6455	0.0
19	99	116	11,948	0.2	Nil	—	—	—	99	116	11,948	0.2
20	63	336	19,241	0.6	4	330	425	78.1	67	336	19,666	2.3
21	30	333	17,086	0.8	1	284	38	28.9	31	331	17,124	0.8
Total or average	1917	312	579,327	2.6	231	296	59,126	52.9	2148	311	638,453	7.3

** Data are given only for trees 15 in. d.b.h. and larger.*

Considerable difficulty was experienced in obtaining adequate samples of all site classes and uniform distributions of age classes within each site class. The natural scarcity of site I together with the necessity of co-ordinating the investigation with current logging, which tends to exclude site V, resulted in an inadequate sample of these two sites. Consequently, only 29 living Douglas firs of site I and 20 of site V were available for analysis, whereas in the samples of sites II, III, and IV there were, respectively, 557, 670, and 641 living firs. Despite the apparently large samples of the intermediate sites, some inadequacies still occurred in these sites with respect to certain age groups.

TABLE III

THE FUNGI ASSOCIATED WITH DECAY OF DOUGLAS FIR IN COASTAL BRITISH COLUMBIA[1]

Species	Type of decay	Volume of associated decay (cu. ft.)	Percentage of total decay volume	Percentage of total infections
Armillaria mellea (Vahl ex Fr.) Quél.	White	97	0.2	0.3
Fomes annosus (Fr.) Cke.	White	192	0.4	1.0
F. officinalis (Vill. ex Fr.) Faull	Brown	883	1.9	1.2
F. pini (Thore) Lloyd	White	8239	17.7	6.7
F. pinicola (Sw.) Cke.	Brown	22,209	47.7	20.4
F. subroseus (Weir) Overh.	Brown	84	0.2	0.6
Ganoderma applanatum (Pers.) Pat.	White	747	1.6	0.9
Merulius himantioides Fries	Brown	1	Tr.[2]	0.1
Odontia bicolor (A. and S. ex Fr.) Bres.	White	Tr.	Tr.	0.1
Omphalia campanella Fries	White	235	0.5	0.1
Peniophora sp.	Unknown	3	Tr.	0.1
Polyporus abietinus Dicks. ex Fries	White	1880	4.0	5.7
P. circinatus Fries	White	3	Tr.	0.3
P. mollis Pers. ex Fries	Brown	Tr.	Tr.	0.1
P. schweinitzii Fries	Brown	5819	12.5	45.7
P. sulphureus Bull. ex Fries	Brown	1489	3.2	4.6
P. volvatus Peck	White	194	0.4	0.2
Poria albipellucida Baxter	White	35	0.1	0.2
P. asiatica (Pilât) Overh.	Brown	35	0.1	0.3
P. carbonica Overholts	Brown	723	1.6	1.3
P. monticola Murr.	Brown	35	0.1	0.4
P. subacida (Peck) Sacc.	White	21	Tr.	0.2
P. weirii Murr.	White	116	0.3	0.7
P. xantha (Fr.) Cke.	Brown	Tr.	Tr.	0.1
Stereum abietinum Pers.	Brown	28	Tr.	0.2
S. chailletii Pers.	White	Tr.	Tr.	0.1
S. pini Fries	White	Tr.	Tr.	0.1
S. sanguinolentum A. & S. ex Fries	White	845	1.8	1.1
Trechispora brinkmanni (Bres.) Rog. and Jacks.	Unknown	Tr.	Tr.	0.1
Unknown	Mixed	2642	5.7	7.1
Total		46,555	100.0	100.0

[1] *Basis: 2148 living and dead trees 15 in. d.b.h. and larger.*

[2] *Trace = less than 1.0 cu. ft. or 0.1%.*

Decays of Douglas Fir

The Fungi Associated With Decayed Wood

Twenty-nine species of fungi caused 912 separate infections in the 2148 living and dead Douglas firs examined in the course of this investigation. An additional 70 infections and 2642 cu. ft. of decay could not be traced to specific fungi. Of the 912 infections and 43,913 cu. ft. of decayed wood traced to specific fungi, five fungi, *Fomes pinicola* (Sw.) Cke., *F. pini* (Thore) Lloyd, *Polyporus schweinitzii* Fries, *P. abietinus* Dicks. ex Fries, and *P. sulphureus* Bull. ex Fries, caused 89% of the infections and 90% of the decay. The relative importance of the different fungi is shown in Table III, which indicates that, in the case of combined living and dead Douglas fir, *Polyporus schweinitzii* may be expected to cause many more infections than either *Fomes pinicola* or *F. pini* but much less decay than either of these two fungi.

Twenty-five different fungi caused 581 separate infections and 14,602 cu. ft. of rot in the 1917 living Douglas firs examined. An additional 42 infections and 628cu. ft. of rot could not be traced to specific fungi. The most important of the fungi found associated with decay of living Douglas fir are listed in Table IV which indicates that, while six different fungi caused significant amounts of decay, most of the decay was caused by *Fomes pini* and *Polyporus schweinitzii*. These two fungi together caused 72% of the infections and 79% of the decay in living fir, *P. schweinitzii* causing far more infections than *Fomes pini* but considerably less decay.

TABLE IV

THE MAJOR FUNGI ASSOCIATED WITH DECAY OF LIVING DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Species	Type of rot	Volume of associated decay (cu. ft.)	Percentage of total decay volume	Percentage of total infections
Fomes pini	White	7467	49.0	9.6
F. pinicola	Brown	505	3.3	4.8
F. officinalis	Brown	314	2.1	1.6
Polyporus schweinitzii	Brown	4582	30.1	62.2
P. sulphureus	Brown	687	4.5	6.4
Poria carbonica	Brown	500	3.3	1.6
Other known fungi	White and Brown	547	3.5	7.1
Unknown	Mixed	628	4.2	6.7
Total		15,230	100.0	100.0

* *Basis: 1917 trees, 15 in. d.b.h. and larger, and 623 infections.*

Sixteen different fungi caused 423 separate infections and 29,311 cu. ft. of rot in the 231 dead Douglas firs examined. An additional 35 infections and 2014 cu. ft. of rot could not be traced to specific fungi. The eight most important fungi found associated with decay of dead fir are listed in Table V which indicates that, of the eight fungi listed, nearly 70% of the rot and nearly half of the infections were associated with *Fomes pinicola*. Of the remaining fungi *Polyporus schweinitzii* and *P. abietinus* were the most important, *P. schweinitzii* causing more infections than *P. abietinus* but less decay. In view of their importance in living trees, a comparison of *Fomes pini* and *Polyporus schweinitzii* in dead trees was made and showed that the average volume of *Fomes pini* decay per infection remained the same in living and dead trees, whereas that of *Polyporus schweinitzii* decay increased from 12 cu. ft. in living trees to 21 cu. ft. in dead trees.

TABLE V

THE MAJOR FUNGI ASSOCIATED WITH DECAY OF DEAD DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Species	Type of rot	Volume of associated decay (cu. ft.)	Percentage of total decay volume	Percentage of total infections
Fomes pini	White	772	2.5	1.7
F. pinicola	Brown	21,704	69.3	47.0
F. officinalis	Brown	569	1.8	0.6
Ganoderma applanatum	White	747	2.4	2.5
Polyporus abietinus	White	1816	5.8	13.5
P. schweinitzii	Brown	1237	4.0	16.7
P. sulphureus	Brown	802	2.6	1.4
Stereum sanguinolentum	White	836	2.6	2.8
Other known fungi	White and Brown	828	2.6	4.1
Unknown	Mixed	2014	6.4	9.7
Total		31,325	100.0	100.0

* *Basis: 231 trees, 15 in. d.b.h. and larger, and 359 infections.*

The Types of Rot

The types of rot were distinguished on the basis of the reactions of their associated fungi in culture in gallic- and tannic-acid media (7, 11). In this manner 46,555 cu. ft. of rot in 2148 living and dead Douglas firs were separated into one or other of three categories; viz., brown-cubical rots, white rots, or mixed and unknown rots (Table VI). Twelve of the different fungi found in living fir caused brown-cubical rots, while the remainder caused white rots.

TABLE VI

THE TYPES OF DECAY OF DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Type of decay	In living trees			In dead trees			In living and dead trees		
	Number of infections	Volume of decay (cu. ft.)	Percentage of total decay	Number of infections	Volume of decay (cu. ft.)	Percentage of total decay	Number of infections	Volume of decay (cu. ft.)	Percentage of total decay
Brown cubical	492	6738	44.2	241	24,568	78.4	733	31,306	67.2
White	88	7864	51.7	83	4743	15.2	171	12,607	27.1
Mixed and unknown	43	628	4.1	35	2014	6.4	78	2642	5.7
Total	623	15,230	100.0	359	31,325	100.0	982	46,555	100.0

** Basis: 1917 living trees, 231 dead trees, all 15 in. d.b.h. and larger.*

The abundance of *Fomes pini* in living Douglas fir resulted in a greater volume of white rot than brown-cubical rot whereas in dead trees, the abundance of *F. pinicola* resulted in a greater volume of brown-cubical rot.

Characteristics of the Major Decays

Fomes pinicola (Figs. 8 and 9) occurred in 9% of the 2148 Douglas firs examined, causing 22,209 cu. ft. of brown-cubical heart rot and sap rot. It was more prevalent in dead trees than in living trees, 70% of the dead trees and less than 2% of the living trees being infected. The average ages of living and dead Douglas firs infected by this fungus were, respectively, 364 and 298 years. The origin of *F. pinicola* infections was traced either to scars, branch stubs, or broken tops, with scars being the most common. Infections originating at branch stubs caused greater average volumes of rot than infections of other origins, being 26 cu. ft. in comparison with 18 cu. ft. for trunk scars, 13 cu. ft. for basal scars, and 5 cu. ft. for broken tops. The average volume of decay in living trees was 17 cu. ft. as compared with 120 cu. ft. in dead trees.

The advanced stage of decay was yellow- to red-brown, friable, cubical, and usually had thick white mycelial mats that filled the shrinkage cracks between the cubes of decayed wood. Wood in an early stage of decay was 'lifeless' in appearance and was usually stained yellow to light-brown. The hard perennial sporophores vary greatly in size and contour. They have a smooth, gray to black upper surface and a red margin that is conspicuous in the case of young sporophores but much less so with old sporophores. The under surface is white to yellow. Sporophores were abundant on dead trees and debris but were totally lacking on living fir.

Fomes pini (Figs. 2, 3, 4, 5) occurred in 3% of the trees examined, the percentage of infection being slightly less in dead trees than in living trees. The 8239 cu. ft. of white-pocket rot ascribed to this fungus occurred only in heartwood, there being no indication that *F. pini* attacks living sapwood despite this phenomenon having been demonstrated by other investigators (5, 12). The average ages of infected living and dead Douglas firs were, respectively, 332 and 289 years. The origins of infection in living trees were traced either to branch stubs or trunk scars, branch-stub infections accounting for over 90% of the rot associated with this fungus. The average volume of decay from both origins remained the same at 124 cu. ft. per infection. More than 70% of the living firs that were infected by *F. pini* bore sporophores, the decay in such trees comprising more than 80% of all the decay caused by this fungus. The average volume of decay in trees having sporophores was 141 cu. ft. and that in trees lacking sporophores was 78 cu. ft. Trees with a single sporophore had, on the average, 81 cu. ft. of rot, those with a single compact cluster of sporophores had 120 cu. ft., and those with vertically-scattered sporophores had 168 cu. ft. (see Table XIII).

The advanced stage of decay occurs as elongated, spindle-shaped pockets that parallel the grain of the wood. The pockets, which are sometimes filled with nearly-pure cellulose, may be separated by apparently sound wood or

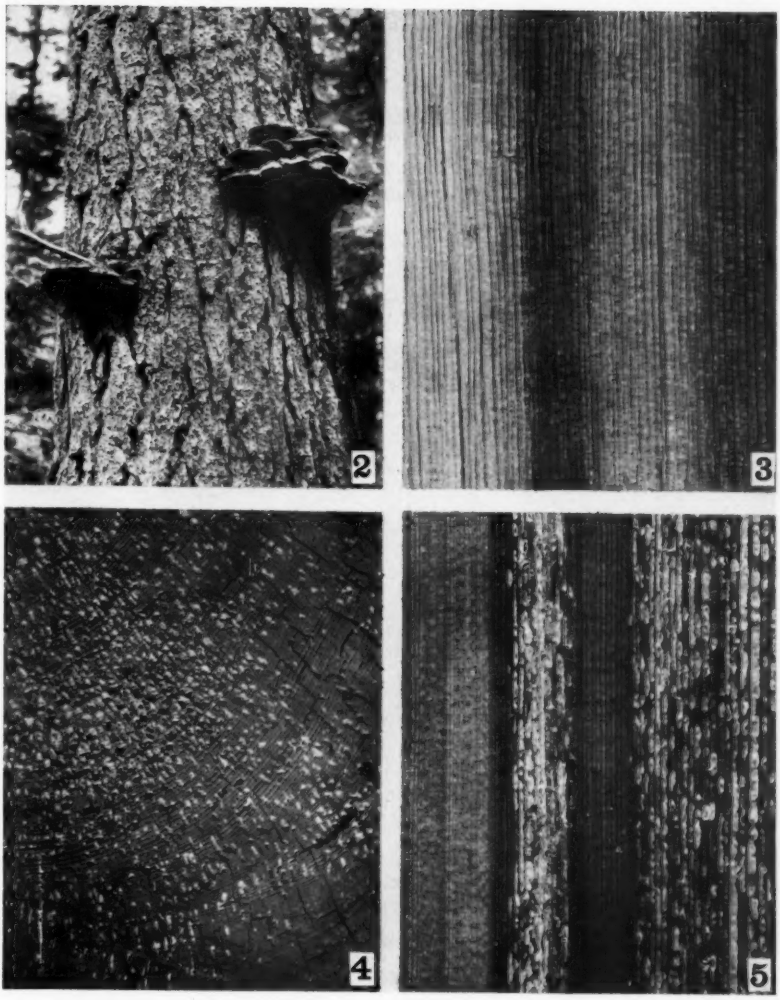

FIG. 2. *Fomes pini:* sporophores.
FIG. 3. *Fomes pini:* incipient decay in radial section.
FIG. 4. *Fomes pini:* advance decay in transverse section.
FIG. 5. *Fomes pini:* advance decay in radial section.

PLATE II

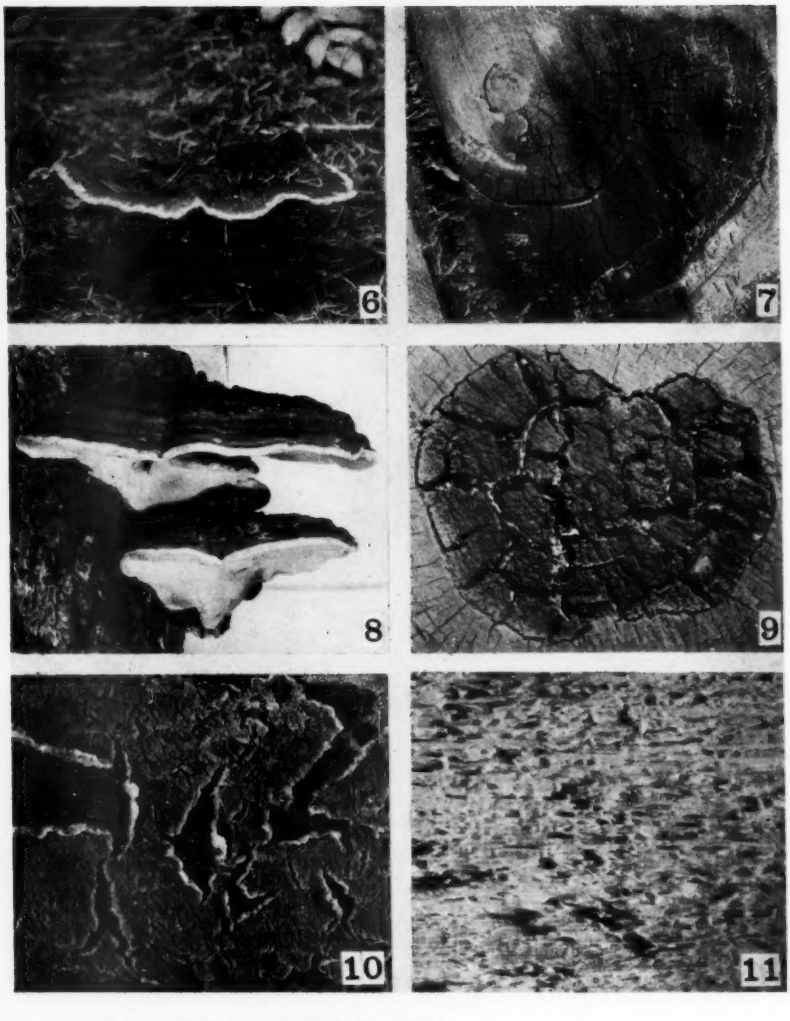

FIG. 6. *Polyporus schweinitzii:* sporophore.
FIG. 7. *Polyporus schweinitzii:* advance decay in transverse section.
FIG. 8. *Fomes pinicola:* sporophores.
FIG. 9. *Fomes pinicola:* advance decay in transverse section.
FIG. 10. *Polyporus abietinus:* sporophores.
FIG. 11. *Polyporus abietinus:* advance decay in tangential section.

they may coalesce. The decay is either uniform throughout a log, restricted to isolated groups of annual rings, or confined to single and widely separated annual rings. Wood in an early stage of decay is red to purple, the discoloration extending several feet beyond advanced rot. The hard, perennial sporophores vary greatly as to size and shape, have a rough, zonate upper surface that is gray to black, and have a brown, velvety margin. The lower surface is gray to brown and has conspicuous, large to small pores.

Polyporus schweinitzii (Figs. 6 and 7) occurred in 21% of the living and dead Douglas firs examined, causing 5819 cu. ft. of brown-cubical heart rot in roots and butts. The percentage of infection was slightly higher in dead trees than in living trees, although dead-tree infections were construed to signify infections in living trees that had subsequently died. Support was lent to this supposition by the fact that the average volume of decay ascribed to *P. schweinitzii* was greater in dead trees than in living trees. The average ages of living and dead trees infected by this fungus remained the same at 314 years. The origins of infection were either roots, basal scars, or trunk scars, root infections being the most common although basal-scar infections caused greater average volumes of decay.

Wood in an advanced stage of decay is red-brown, friable, and cubical. The cubes of decayed wood are separated by shrinkage cracks that are sometimes filled with white mycelial mats or resin deposits. Wood in an early stage of decay is discolored to varying shades of brown and yellow. The annual sporophores grow on the ground from decayed roots, at log ends, and on exposed wood of basal scars. They are thin, fleshy when fresh, imbricate when growing on trees and logs, and circular and stipitate when growing on the ground. The dark-brown upper surface is velvety and has a yellow margin. The lower surface is yellow-green and bears shallow irregular pores. Sporophores occurred on very few of the trees examined.

Polyporus abietinus (Figs. 10 and 11) occurred in 3% of the living and dead trees examined, causing 1880 cu. ft. of white-pocket rot in dead sapwood. It was of no practical importance in living fir, being associated in all cases with the dead sapwood of wounds. The average volumes of decay caused by this fungus in living and dead trees were, respectively, 11 and 37 cu. ft. Advanced decay occurs as a close network of fine pockets that are at first filled with white deposits but which are later empty. Incipient decay occurs as yellow or brown discolorations. The small annual sporophores are resupinate to effused-reflexed and have a hairy, zonate, grayish upper surface. The lower surface is pink to brown and has shallow irregular pores. Sporophores were common on dead trees and debris but rare on living trees.

Decay of Living Douglas Fir

Decay in Relation to Tree Age

An examination of 1910 Douglas firs showed that both the percentage of trees having decay and the average volume of decay per tree increased with increasing tree age. The influence of site on decay incidence was consistent

for most of the age groups sampled, the percentage of trees infected increasing with increasing site quality. Thus, the percentage of living firs infected on sites IV, III, and II was, respectively, 23.1, 30.2, and 45.7. Likewise, the average volume of decay, although consistently low, increased with increasing site quality. The average volume of decay per tree on sites IV, III, and II was, respectively, 3, 7, and 10 cu. ft. When only the infected trees were considered, the average volume of decay on sites IV, III, II was, respectively 12, 25, and 39 cu. ft. per tree. The low volumes of decay encountered, together with the high gross volumes characteristic of Douglas fir, resulted in very low losses, irrespective of the age or site class investigated. Consequently on all sites and in all age classes up to 400 years, gross increment exceeded decay increment with very few exceptions. In contrast with the percentage of infection and average volume of decay, both of which increased regularly with increasing tree age and site quality, the percentage loss from decay increased to a maximum value on sites III and II and declined thereafter. On site IV land, the percentage loss increased regularly with increasing tree age. The maximum loss on sites IV, III, and II was, respectively, 3.8% at 370 years, 5.2% at 280 years, and 7.4% at 270 years. The percentage loss for the combined age groups on sites IV, III, and II was, respectively, 2.2, 2.7, and 2.8. Thus, decay was of nearly equal importance on all sites but appeared to become most important earlier on good sites than on poor sites.

TABLE VII

THE RELATION BETWEEN DIAMETER AND DECAY LOSS IN LIVING DOUGLAS FIR

(Curved values)*

D.b.h. (in.)	Site			
	IV	III	II	V–I
	Percentage of the gross merchantable volume decayed			
10	—	—	0.8	—
15	0.1	0.2	1.9	0.1
20	0.5	0.8	2.9	0.6
25	1.0	1.4	3.4	1.3
30	1.9	2.2	3.6	2.3
35	3.1	3.2	3.5	3.3
40	4.6	4.2	3.3	3.7
45	6.8	4.5	3.1	3.7
50	8.4	3.5	2.9	3.5
55	—	2.3	2.6	3.1
60	—	1.4	2.4	2.6
65	—	—	2.1	2.1
70	—	—	1.8	1.6
75	—	—	1.6	1.2
80	—	—	1.3	0.7

* *Based on 640 site IV, 556 site III, 485 site II, and 1729 sites V–I trees.*

Decay in Relation to Tree Diameter

An examination of 1729 Douglas firs showed that the percentage of trees infected and the average volume of decay per tree increased with increasing tree diameter on all sites. The increase in percentage of infection for the combined sites was rapid at first, being 20% at 20 in. d.b.h. and 44% at 40 in., but increased more slowly in the larger diameters. The average volume of decay per tree for the combined sites increased from 1 cu. ft. at 20 in. d.b.h. to 31 cu. ft. at 80 in. Trees of the same diameter were more likely to be infected and to have greater amounts of decay as site quality increased, particularly in the case of small-sized trees. As trees increased in size, however, the differences in the percentage of trees infected and the average amount of decay per tree became less pronounced between sites. The percentage of the gross volume of trees decayed increased regularly with increasing tree diameter on site IV land but increased to a maximum on sites III and II, declining thereafter (Table VII). In general, the percentage loss in small-sized trees was greater on good sites but, as trees increased in size, the losses on poor sites exceeded those on good sites.

Decay in Relation to Site

As analysis of the relation of site to the occurrence and amount of decay associated with the six most important fungi that occurred in living Douglas fir showed that, without exception, both the incidence of infection and average volume of decay increased with increasing site quality (Table VIII). Consequently, from the aspects of occurrence and amount of decay, it is evident

TABLE VIII

THE RELATION OF SITE TO THE OCCURRENCE AND AMOUNT OF DECAY CAUSED BY DIFFERENT FUNGI IN LIVING DOUGLAS FIR IN COASTAL BRITISH COLUMBIA

Fungus	Site							
	IV	III	II	V–I	IV	III	II	V–I
	Percentage of trees infected				Average volume of associated decay (cu. ft.)*			
Polyporus schweinitzii	18.4	21.5	27.2	22.3	7	10	18	12
Fomes pini	1.3	3.8	6.0	3.2	86	107	146	131
Polyporus sulphureus	1.9	1.4	3.9	2.3	5	14	26	17
Fomes pinicola	0.3	0.7	3.9	1.5	6	6	20	17
Poria carbonica	0.2	0.5	1.2	0.6	22	32	64	50
Fomes officinalis	0.5	0.4	1.0	0.6	8	19	51	31
Other fungi	0.5	1.9	2.5	1.8	—	—	—	—
All fungi	23.1	30.2	45.7	32.3	11	25	39	27

* *The volumes shown are those associated with only the fungus indicated.*

that Douglas fir on good sites may be expected to be more heavily infected and to have larger amounts of decay than on poor sites. However, the increased amount of decay on good sites may be expected to be more than offset by the increased gross volume of trees on such sites.

Decay in Relation to Latitude

In view of the increased amount of decay reported for Douglas fir in Washington, Oregon, and California (4, 5, 9) over that which appears to occur in British Columbia, an examination of the Vancouver Island samples was made to determine whether or not decay could be related to latitude. Accordingly, three regions were recognized for Vancouver Island; viz., southern, central, and northern. The examination suggested that, despite the generally small amounts of decay in all three regions, decay may be expected to decrease with increasing latitude (Table IX). It should be noted, however, that high-defect and low-defect stands may be anticipated in all regions.

TABLE IX

THE RELATION OF LATITUDE TO DECAY OF DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Region	Number of plots	Number of trees	Average age of trees	Average site	Percentage of trees infected	Percentage of gross volume decayed
South	30	459	287	III	30.3	2.8
Central	71	1026	313	III	29.6	2.1
North	14	83	381	II	20.4	0.5
All regions	115	1586	309	III	29.3	2.0

* *The difference in latitude between the most southerly and northerly samples was 120 miles.*

Decay in Relation to Avenue of Entrance

An examination of 1917 living Douglas firs revealed five categories of infection courts; viz., roots, basal scars, branch stubs, trunk scars, and broken tops. Table X shows for all fungi, and in particular for *Polyporus schweinitzii* and *Fomes pini*, the relative importance of each category. Root infections were greatest in number but branch-stub infections caused the highest average volumes of decay. Most root infections were caused by *Polyporus schweinitzii* while most branch-stub infections were caused by *Fomes pini*. Consequently, by virtue of their great frequency and despite their low average volumes of decay, root infections of *Polyporus schweinitzii* caused 77% of the decay traced to this fungus and 23% of all the decay encountered. Branch infections of *Fomes pini*, by virtue of their high average volumes of decay and despite their low frequency, caused nearly all of the decay traced to this fungus and almost half of all the decay recorded in living trees.

TABLE X

THE RELATIVE IMPORTANCE OF DIFFERENT INFECTION COURTS TO DECAY OF DOUGLAS FIR IN COASTAL BRITISH COLUMBIA*

Infection court	*Polyporus schweinitzii*				*Fomes pini*				All fungi			
	Infections		Decay		Infections		Decay		Infections		Decay	
	Number	Percentage of total	Volume (cu. ft.)	Percentage of total	Number	Percentage of total	Volume (cu. ft.)	Percentage of total	Number	Percentage of total	Volume (cu. ft.)	Percentage of total
Roots	319	82.2	3506	76.6	—	—	—	—	386	61.9	4405	28.9
Basal scars	68	17.5	1038	22.6	—	—	—	—	104	16.7	1450	9.5
Branch stubs	—	—	—	—	56	93.3	6976	93.4	89	14.3	8144	53.5
Trunk scars	1	0.3	38	0.8	4	6.7	491	6.6	36	5.8	1164	7.7
Broken top	—	—	—	—	—	—	—	—	8	1.3	67	0.4
Total	388	100.0	4582	100.0	60	100.0	7467	100.0	623	100.0	15,230	100.0

** Basis: 1917 trees.*

TABLE XI

THE COMPARATIVE EFFECTIVENESS OF TREE ABNORMALITIES AS INDICATORS OF DECAY IN LIVING DOUGLAS FIR

Type of indicator	Number observed*	Number with decay (%)	Volume of associated decay (cu. ft.)	Percentage of total decay volume	Average volume of decay per infection (cu. ft.)	Average volume of decay per observation
Sporophores	56	100.0	6695	43.9	120	120
Swollen knots	7	100.0	636	4.2	91	91
Swollen butt	13	69.2	406	2.7	45	31
Leaning tree	8	62.5	129	0.8	26	16
Branch stubs	181	49.2	1989	13.1	22	11
Broken top	24	33.3	100	0.7	13	4
Basal scar	349	29.9	1232	8.1	12	3
Trunk scar	122	29.5	753	4.9	21	6
All types	760	41.3	11,940	78.4	38	16

* *The number of trees having one or more of the abnormality noted.*

TABLE XII

THE SIGNIFICANCE OF SPOROPHORES AS INDICATORS OF DECAY CAUSED BY *Polyporus schweinitzii* AND *Fomes pini* IN LIVING DOUGLAS FIR

Method of decay entrance	Number of trees infected	Volume of decay	Percentage of infected trees having sporophores	Percentage of decay volume indicated by sporophores	Average volume of decay in trees having sporophores	Average volume of decay in trees lacking sporophores
Polyporus schweinitzii						
Roots	319	3506	2.2	11.2	56	10
Basal scar	68	1038	2.9	4.0	21	15
Trunk scar	1	38	0.0	0.0	—	38
Total or average	388	4582	2.3	9.5	48	11
Fomes pini						
Trunk scar	4	491	50.0	97.1	238	7
Branch stubs	56	6976	75.0	82.3	137	88
Total or average	60	7467	73.3	83.3	141	78

Decay in Relation to External Indicators

Eight of the many different types of tree abnormalities noted appeared to be effective as indicators of hidden decay. Trees having one or other of the indicators listed in Table XI were examined in order that the comparative effectiveness of each type of indicator could be rated. Since sporophores and swollen knots proved to be the only positive indicators, together accounting for nearly half of the decay in living Douglas firs, further analyses of trees with these indicators were made to learn in more detail their significance in estimating decay in Douglas-fir stands. The results of these analyses are shown in Tables XII and XIII which indicate that, in general, trees with sporophores are more decayed than trees lacking sporophores. It was apparent also that sporophores of *Polyporus schweinitzii*, being associated with less than 10% of the decay ascribed to this fungus and less than 3% of all the decay in living trees, cannot be accorded much practical significance in the evaluation of decay in stands. In contrast, the sporophores and swollen knots of *Fomes pini* appear to have considerable diagnostic value with respect to decay in stands. Likewise, the number and distribution of *F. pini* sporophores appear to indicate the relative amounts of decay in individual trees.

TABLE XIII

THE SIGNIFICANCE OF SPOROPHORES AND SWOLLEN KNOTS OF *Fomes pini* IN ESTIMATING DECAY OF LIVING DOUGLAS FIR

Type of indicator	Number of trees	Volume of associated decay	
		Total (cu. ft.)	Average (cu. ft.)
Sporophores lacking	9	702	78
Single sporophore	9	731	81
Swollen knots	7	636	91
Single cluster of sporophores	8	961	120
Sporophores vertically scattered	27	4527	168
Total or average	60	7467	125

Decay of Dead Douglas Fir

An examination of the 231 dead Douglas firs in the total sample of 2148 trees showed that, although more than half of the decay recorded for the entire sample occurred in dead trees, many of them were more than 50% sound. The occurrence of dead Douglas firs for the different sites is shown in Table XIV, which indicates that considerable variation may be expected in the condition of dead trees according to the site quality of the stand in which they occur. The examination showed that, in general, the percentage

TABLE XIV

THE RELATION OF SITE TO THE OCCURRENCE AND CONDITION OF DEAD DOUGLAS FIR IN COASTAL BRITISH COLUMBIA

Site class	IV	III	II	V–I
Number of trees	46	93	83	231
Percentage of total trees	6.7	11.8	12.5	10.8
Average age (yr.)	285	272	298	296
Average diameter (in.)	22	29	36	31
Average gross volume (cu. ft.)	78	197	397	251
Average decay volume (cu. ft.)	62	113	183	133
Average loss (%)	79.2	56.9	46.2	53.0
Percentage of trees merchantable*	37.0	39.8	56.6	43.3

* *Percentage of the dead trees that were at least 50% sound.*

loss in dead trees for the combined sites decreased with increasing tree size, trees 20 in. d.b.h. being 68% decayed and trees 60 in. d.b.h. being only 32% decayed.

The proportion of merchantable trees[12] in the dead-tree class, although less than half for the combined sites, increased with increasing site quality to more than half in site II stands. On the basis of a comparison drawn between merchantable and non-merchantable dead trees it appeared that, for trees of the same age, merchantable trees were larger and contained smaller average volumes of decay than non-merchantable dead trees. Thus, merchantable dead trees for the combined sites were, on the average, only 28% decayed whereas non-merchantable dead trees were 78% decayed.

Discussion

The present investigation was initiated on request of British Columbia lumbering interests and the provincial forest service to provide information relative to the pathology of old-growth Douglas fir in coastal British Columbia. These agencies made their requests in the light of discrepancies between the usual conditions experienced by them, with respect to decay of fir, and the results of earlier investigations made in the Pacific Northwestern United States.

The results of the British Columbia investigation present a strong indication that decay of old-growth Douglas fir is of much less consequence in this province than it has been shown to be farther south. The investigation

[12] *Trees at least 50% sound.*

revealed that, although an average loss from decay of 2.6% may be expected for living fir in coastal British Columbia, greater losses may be anticipated in some stands. Likewise, while the proportion of living firs infected was less than half and the average volume of decay per tree was low, greater intensities of infection and higher volumes of decay were found in some trees and stands. However, the occasional high losses found to occur in individual trees or stands were, in many cases, detected in standing timber by associating unusual amounts of decay with one or other decay indicator. Thus, 73% of the trees infected by *Fomes pini* and 83% of the decay ascribed to this fungus were revealed by the occurrence and location of sporophores and swollen knots. Furthermore, the volume of decay so indicated amounted to 41% of all the decay recorded for living fir.

Although 25 different fungi were isolated from decayed wood in living firs and 29 in living and dead trees combined, the maximum loss from any one fungus was 1.3% of the gross merchantable volume for living trees and 3.5% for living and dead trees combined. The maximum loss in living trees was caused by *Fomes pini*, while that in living and dead trees combined was caused by *Fomes pinicola*. A comparison of *Fomes pini* in living and dead Douglas firs showed that its incidence decreased slightly from living trees to dead trees while the average amounts of *F. pini* decay remained the same. In contrast, the incidence of *Polyporus schweinitzii* increased slightly from living trees to dead trees and the average amount of this type of decay almost doubled. Thus, of the two fungi, *P. schweinitzii* appeared to be more closely associated with mortality of Douglas fir than did *F. pini*.

The irregularities found in the occurrence of decay-producing fungi and the amounts and types of decay in Douglas fir may be partially explained by the influences of site, tree age or size, latitude, and stand history. Thus, while the percentage of trees infected and average amount of decay for living trees increased with increasing site quality, tree age, and tree size, the percentage loss from decay increased to a maximum with increasing tree age or size and declined thereafter for most sites. Despite the larger amounts of decay that occurred on good sites, the percentage loss for the combined age or size classes was approximately equal on all sites by virtue of the higher average gross volumes characteristic of trees on good sites. The indicated decrease in the extent of decay loss concurrent with increasing latitude was rendered uncertain by the small amounts of decay that occurred throughout coastal British Columbia. However, should the British Columbia sample be considered in its entirety and compared with samples of Douglas fir from Washington, Oregon, and Northern California, decreased amounts of decay with increasing latitude become more apparent. The reduced importance of decay in Douglas fir in its northern latitudes does not, however, remove the possibility of occasional high losses occurring anywhere within the range of Douglas fir. Stand history can play a significant role in decay of Douglas fir as evidenced by the association of fires with much of the decay that occurred in the most defective stands sampled. It appears, therefore, that in coastal

British Columbia, where average conditions of decay in Douglas fir appear to be of little consequence, instances of excessive amounts of decay are often likely to be related to stand disturbances, such as fires, that have occurred early in the course of stand development.

Acknowledgments

The authors are indebted to numerous persons for advice and assistance in the course of the investigation. Appreciation is expressed, in particular, to Dr. R. E. Foster for his counsel and active interest in the work; to Mr. A. W. McCallum for his critical reading of the manuscript; and to the foresters and other senior employees of British Columbia Forest Products Limited, Canadian Forest Products Limited, Hillcrest Lumber Company Limited, MacMillan and Bloedel Limited, and Pacific Mills Limited, all of whom, by making available their company lands and by co-operating in many other ways, made specific and important contributions to the work.

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SEED DEVELOPMENT IN BLACKBERRIES[1]

By Ernest A. Kerr[2]

Abstract

This study of blackberries deals with seed production and germination, describes the main steps in megagametophyte development of pseudogamous and syngamous varieties, and traces the development of both germinable and aborted seeds. Certain conclusions are drawn concerning the interdependence of maternal and filial tissues. The number of drupelets which develop in both pseudogamous and syngamous varieties depends, in part, on the pollen parent. A small but rather constant proportion of the drupelets display only a partial enlargement. Seeds of the well-developed drupelets are divided into three intergrading classes: (i) plump seeds, (ii) collapsed seeds, and (iii) an intermediate group. The general developmental features of the ovule and its contents are described. Regular megagametophyte formation is the rule in the syngamous varieties, but many irregularities, especially multiple gametophytes, are common in the pseudogamous species studied. Approximately a third of the seeds in the drupelets which appeared normal at 6 to 15 days after pollination did not contain an embryo. Some of the "eggs" were disintegrating, whereas others were still in a turgid condition. All the seeds which were imperfectly developed contained endosperm but only rarely was there an embryo or its remains. Reserve food was usually stored only in the chalazal region of the endosperm. The endosperm is believed to stimulate the transfer of nutrient materials to the seed. The embryo supplements this function as the seed approaches maturity, and influences the distribution of the storage products within the endosperm.

Introduction

The genus *Rubus* offers good material for a study of the interrelationships of the embyro, endosperm, and maternal tissues. Nonfunctional ovules are often formed, and frequently both seeds and drupelets which begin development fail to mature normally. Commercial varieties usually produce sufficient functional ovules to ensure an abundant crop under favorable conditions.

The blackberry fruit is an aggregation of drupelets which develop independently while adhering to a common receptacle. Although seed abortion frequently takes place there are no "drops" of the individual blackberry ovaries such as occur in the rosaceous fruit-trees (9, 16, 17). Hence, the drupelets corresponding to those which would drop in plums or peaches can be studied at all stages up to maturity. It is possible that the aborting drupelets in *Rubus* might develop for a longer period than in *Prunus* but the general picture is similar.

Both syngamic and pseudogamic forms of *Rubus* are available for comparison. Fertilization of both the egg and the two polar nuclei takes place in syngamic seed formation. Only the two polar nuclei are fertilized in pseudogamic seed development: the egg develops parthenogenetically (20). Hence the only genetic variable of the embryo–endosperm-maternal tissue

[1] *Manuscript received June 23, 1954.*

Paper No. 525 from the Department of Genetics, Wisconsin Agricultural Experiment Station, University of Wisconsin, Madison, Wis., U.S.A. This paper is part of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Genetics, University of Wisconsin. The work was supported in part by a grant from the Research Committee of the Graduate School with funds from the Wisconsin Alumni Research Foundation.

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complex in pseudogamic forms is the endosperm. Christen (2) made a comprehensive study of the origin of the megagametophyte in pseudogamic and syngamic species of *Rubus*, but postpollination studies have been fragmentary (21).

Materials and Methods

Three facultatively pseudogamic varieties of *Rubus*—Evergreen, Himalaya, and Brainerd (8)—and five syngamic varieties—Eldorado, BB-1, BB-2, and two native raspberry species (14)—were used in the present investigation (Table I). The blackberry varieties BB-1 and BB-2, as well as the red and black raspberries, were from volunteer clones growing in the vicinity of Madison, Wis. The others were commercial varieties. All of the plants appeared to be free from disease. Examination of blackberry fruits from two locations in Ontario confirmed the macroscopic studies herein reported.

The flowers were emasculated approximately one day before they would normally open. Satisfactory protection from unintentional pollination was obtained by wrapping a small piece of pliofilm firmly around each flower and sealing it with cellulose tape. This protector was replaced following pollination one or two days later.

The histological material was fixed in Carnoy's 3 : 1 : 1 for one hour, then washed in 80% ethyl alcohol and stored in 70% alcohol. The seeds were dissected from the drupelets while in the 70% alcohol and then run through a long alcohol–cedarwood oil–paraffin series in order to secure satisfactory penetration of the paraffin. Sections were cut at 15 microns. Various combinations of stains were used. A combination of Delafield's haematoxylin and safranin proved to be the most satisfactory for early stages of seed development. A safranin–fast green combination was better for mature seeds. All drawings were made with the aid of a camera lucida.

Seed Germination

The best germination of blackberry "seed" has heretofore been about 35% (12). A means of obtaining 100% germination was desired to determine whether the embryos in those seeds which do not normally germinate were hybrids or apomicts. Samples of BB-1 nutlets were given various mechanical, chemical, and thermal treatments. The most satisfactory germination was obtained after the endocarps had been removed by means of tweezers and triangular needles, and the seeds stored on moist blotting paper for five months at 2°–3° C. The two lots of 25 and 40 blackberry seeds getting this treatment gave 88% and 90% germination. The rest of these seeds decayed.

Drupelet Production

The number of ovaries per flower was determined for the different species in order to get a check on the efficiency of pollination. The data were obtained by counting the number of ovaries at the open-flower stage and the number of drupelets at varying stages in the development of the aggregate

TABLE I

NUMBER OF OVARIES PER FLOWER IN CERTAIN VARIETIES OF *Rubus*

Variety	Species	No. of flowers	No. of ovaries	Av. no. ovaries
Evergreen	*R. laciniatus* Willd.	338	18,835	55.5
Himalaya	*R. procerus* P. J. Muell.	125	8,518	68.1
Brainerd	Himalaya × Georgia Mammoth (?)	71	5,939	83.6
BB-1 and BB-2	*R. allegheniensis* Porter	351	29,309	83.5
Eldorado	*R. allegheniensis* Porter × *R. argutus* Link (?)	105	10,278	97.9
Red raspberry	*R. strigosus* Michx.	79	3,683	46.6
Black raspberry	*R. occidentalis* L.	295	14,104	47.8

TABLE II

NUMBER OF DRUPELETS DEVELOPING AFTER CERTAIN POLLINATIONS

Cross	No. fruits	Av. no. drupelets	Per cent well developed drupelets	Per cent poorly developed drupelets	Per cent undeveloped drupelets
Evergreen × open	70	56.9	33.7	6.2	60.1
Evergreen × self	33	54.7	25.7	3.5	70.8
Evergreen × Brainerd	12	54.6	36.9	3.1	61.0
Evergreen × Himalaya	10	58.5	28.5	9.6	61.9
Evergreen × BB-1	25	56.8	21.9	4.5	73.6
Evergreen × red raspberry	21	65.9	0.7	2.0	97.3
Evergreen × black raspberry	13	53.1	0.9	3.8	95.3
Evergreen × *Rosa rugosa* Thunb.	2	46.0	0.0	0.0	100.0
Evergreen unpollinated	111	55.9	0.0	0.3*	99.7
Himalaya × self	13	62.2	39.1	11.0	49.9
Himalaya × Brainerd	9	70.7	34.6	5.3	60.1
Himalaya × Evergreen	9	76.1	36.4	10.4	53.2
Himalaya × BB-1	8	67.9	53.2	6.6	40.2
Himalaya × red raspberry	9	66.6	8.3**	4.3	87.4
Himalaya × black raspberry	5	72.0	0.8	4.7	94.5
Himalaya × *Rosa rugosa* Thunb.	11	69.6	0.0	3.1	96.9
Himalaya unpollinated	23	66.2	0.0	0.2	99.8
BB-1 × open	60	96.0	53.6	3.8	42.6
BB-1 × self	9	80.2	55.5	1.0	43.5
BB-1 × Eldorado	13	77.1	45.7	1.7	52.6
BB-1 × Evergreen	5	104.2	15.0	6.1	78.9
BB-1 × red raspberry	15	81.1	3.9	1.0	95.1
BB-1 × black raspberry	8	83.8	1.3	0.3	98.4
BB-1 unpollinated	5	75.2	0.5	0.5	99.0
Black raspberry × open	168	48.7	80.3	1.3	18.4

* *One fruit produced 69% of the poorly developed drupelets.*
** *One fruit produced 72% of the well developed drupelets.*

fruit. Ovary production in *Rubus* is influenced by several factors: (1) the species (Table I), (2) the position of the inflorescence on the cane, (3) the position of the flower on the inflorescence (the terminal flower usually possesses more ovaries than any of the others), and (4) the external environment such as soil and weather.

Data were collected on the number of drupelets which were developing at various intervals (six days to maturity) after certain pollinations (Table II). One column of the table shows the percentage of poorly-developed drupelets. These were plump and appeared normal in every respect except that they were small. Until six days after pollination there was an intergradation between the three types of drupelets—undeveloped, poorly developed, and well developed.

The above data indicate a high degree of sterility in the blackberries studied even though the fruits, at maturity, were typical of commercial varieties in size, shape, and appearance. Similar results were obtained by Peitersen (22) who reported that *R. allegheniensis* had only 36% of the ovaries developing after open pollination. Since the low drupelet-set might have been an indication that all the megagametophytes were not at the same stage of maturity at the time the single pollination was made, a few emasculated flowers were pollinated several times over a period of three days. No increase in number of developing drupelets was obtained.

Seed Production in Well-developed Drupelets

The nutlets contained in all well-developed drupelets were, except for some slight variation in size and shape, externally similar (Fig. 1). Over half of the seeds were plump and apparently capable of germinating (Fig. 2). The seeds in many of the normal-appearing nutlets, however, were completely collapsed (Fig. 3). They had grown to the usual volume and then had shrivelled to flat, or saucer-shaped remnants slightly shorter and narrower than the ovarian cavity. There were also many seeds which showed intermediate degrees of filling (Fig. 4). The great majority of these seeds were plump at the chalazal end and collapsed at the micropylar end with a distinct line of demarcation between the two regions. A few were plump at both ends and collapsed in the middle. Table III gives, for various crosses, the percentage of seeds showing partial or total collapse. As high as 67% of the nutlets which appeared normal contained grossly defective seeds. Some of the nutlets floated when put in water. All of these contained seeds which had shrunken to some extent. The number of defective seeds in the nutlets which sank in water varied from 0% for the cross Himalaya × Evergreen to 20.5% for the cross Evergreen × Brainerd. The nutlets of all the hybrids and polyploids had a high proportion of these defective seeds. Only the fruits of the diploid raspberry had the seed-set which normally would be expected. Similar defective seeds have been noted by Crane and co-workers following the pollinations *R. procerus* selfed (4) and *R. rusticanus inermis* × *R. procerus* (6).

TABLE III

PERCENTAGE OF SEEDS SHOWING PARTIAL OR TOTAL COLLAPSE IN NUTLETS WHICH APPEARED NORMAL

Cross	Number of nutlets	Per cent collapsed seed
Evergreen × open	582	49.5
Evergreen × self	37	35.1
Evergreen × Himalaya	115	65.2
Evergreen × Brainerd	131	56.3
Evergreen × BB-1	212	47.7
Total Evergreen	1077	51.2
Himalaya × Evergreen	133	35.3
Himalaya × BB-1	162	43.8
Total Himalaya	295	40.0
BB-1 × open	198	25.8
BB-1 × Eldorado	313	29.4
Total BB-1	511	28.0
Red raspberry × open	151	1.3

Histological Studies

Syngamous Blackberries

Very little difference could be detected in the ontogeny of the seeds of the Eldorado, BB-1, and BB-2 varieties. The observations recorded here were on Eldorado for prepollination stages and on BB-1 for postpollination stages.

Two ovules, one slightly above the other, begin development in the upper part of the ovarian cavity. One of these usually aborts after the megaspore mother-cell differentiates but before the gametophyte matures. The upper ovule reached maturity in only one out of 50 ovaries examined for the points of insertion of the funiculi. Apparently the lower ovule, by its proximity to the nutrient supply, has a distinct advantage. Frequently ovules fail to develop in one or two of the ovaries at the top of the flower and both ovules may abort before maturity.

Megasporogenesis in the Eldorado variety was generally similar to that in *R. fruticosus* (21) and *R. tomentosus* (2). Usually one archesporial cell of the fertile subepidermal region of the nucellus gains the ascendancy at the time

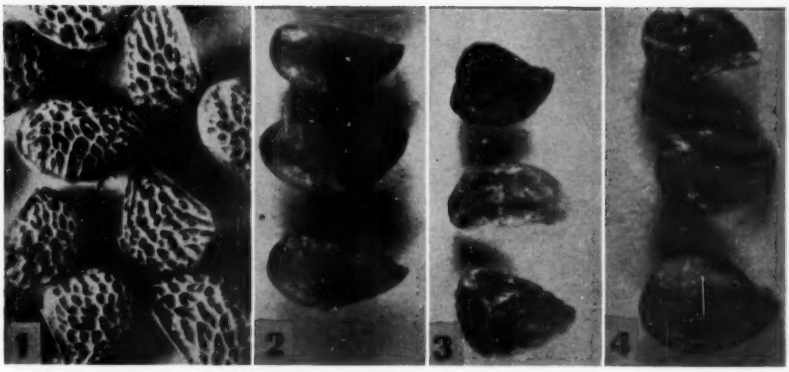


FIG. 1. Normal nutlets of BB-1 blackberry showing well-developed ridges of the endocarp. There is some variation in size and shape of the nutlets (× 8).
FIG. 2. Plump BB-1 seeds capable of germinating (× 12).
FIG. 3. Completely collapsed BB-1 seeds. These were obtained from well-developed nutlets (× 12).
FIG. 4. BB-1 seeds showing various degrees of collapse. Most of these seeds are plump at the chalazal end and collapsed at the micropylar end (× 12).

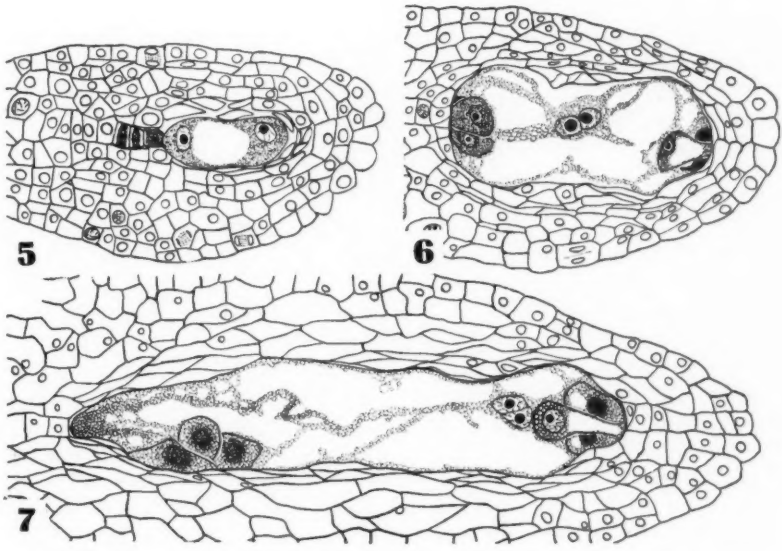


FIG. 5. Binucleate Eldorado megagametophyte developing from the micropylar megaspore (× 365).
FIG. 6. Eldorado megagametophyte showing the two polar nuclei meeting in the center of the cell (× 365).
FIG. 7. BB-1 megagametophyte one day after pollination. The polar nuclei are in the region of the egg, the antipodals are beginning to disintegrate, and the cytoplasm of the central cell is concentrated at its chalazal end (× 365).

the single integument has reached the top of the nucellus. Reduction division and the production of megaspores frequently, if not always, takes place as is indicated in Fig. 5. The linear position of the spore which continues development is variable, but megagametophyte formation is of the normal type (19). The two polar nuclei become closely appressed to each other near the center of the endosperm mother-cell (Fig. 6), then move towards the egg. This movement appears to be greater than it actually is because the chalazal end of the gametophyte is extending rapidly at this time. The three antipodals are disintegrating at the mature gametophyte stage (Fig. 7) and are frequently located a short distance from the chalazal end. The single integument has grown over the nucellus so that the micropyle is closed and the nucellar cap has reached its maximum thickness of five to seven cells.

Maturation of the megagametophyte usually coincides with the opening of the flower. However, exceptions to this are frequently found. The megagametophytes in two ovules 24 hr. after pollination were still in the binucleate stage and in one instance, two days after pollination, an enlarged megaspore was observed. Gametophytes with four or eight free nuclei are fairly common at the open-flower stage. Every stage from megaspore to mature megagametophyte may be found in one flower.

One gametophyte generally develops in each ovule, but occasionally two may be formed. These are usually in contact laterally, but in one ovule they were located end to end and separated by several cells.

Double fertilization takes place about a day after pollination. The primary endosperm nucleus then moves to the center of the endosperm mother-cell where it divides about a day later. The zygote usually divides three or four days after pollination when the endosperm has 16 to 32 free nuclei. The male and female components of the zygote can still be identified at late prophase of the first division (Fig. 11). The zygote first forms a linear row of cells of which the terminal one gives rise to the embryo proper. The cotyledons differentiate about 15 days after pollination. The free-nuclear endosperm is at first concentrated at the micropylar end and to a less extent at the chalazal end. The nuclei are small in the densely staining cytoplasm at the chalazal end but are much larger and divide more frequently at the distal end. Only a thin layer of cytoplasm with a few nuclei is found along the sides of the endosperm. The whole cavity is gradually filled with protoplasm as the seed grows older. Cell-wall formation begins at the micropylar end of the endosperm sometime between 10 and 15 days after pollination, and progresses toward the chalazal end.

Twin embryos were observed in single endosperms of two seeds (Fig. 12). Both cases were similar. The larger embryo had nuclei which were approximately twice the volume of those of the smaller one. This indicates that the larger embryo was diploid and the smaller one haploid. Both the embryos had a maximum of two nucleoli per nucleus. The presence of a second nucleolus in the smaller embryo is probably attributable to the polyploid nature of the parent.

The ovule has a distinctive vascular development. A single vascular bundle extends from the funiculus to the chalazal end of the anatropous ovule where it forms a saucer-shaped terminus. This region is filled with a somewhat irregular mass of cells whose protoplasm contains large vacuoles. The protoplasm in material collected five days after pollination adhered closely to the cell walls which did not stain with Delafield's haematoxylin. Above this discus-shaped layer, there is a region of very densely cytoplasmic, thin-walled cells. This region extends from the line of separation between the integument and the base of the nucellus up towards the center of the ovule. The cells are orientated in a longitudinal direction, indicating that they are instrumental in the direct transfer of nutrients from the vascular bundle to the gametophyte. The densely cytoplasmic region changes more or less abruptly to one of thin-walled cells with scant contents. It extends as far as the megagametophyte. The cell walls nearest the endosperm have collapsed and are being digested. The lower cells of this strand show much mitotic activity and are steadily replacing those that are disintegrating. In median section, at one day after pollination (Fig. 8) the lowest region (vacuolate) is approximately two cells thick, the central one (cytoplasmic) 12 cells thick, and the upper one (disintegrating) absent. At five days, these regions are about three, eight, and two cells thick respectively, and the cytoplasmic cells of the whole chalazal region are richly supplied with ergastic materials. By 15 days (Fig. 9) the regions are approximately five, three, and four cells thick. The developing endosperm resorbs the nucellar cells at the sides of this strand faster than those of the strand itself. In the mature seed the cytoplasmic region is lacking and the innermost region is reduced to a layer of crushed cell-walls. The vacuolate region (Fig. 10) is divided into three layers each about two cells thick. The basal layer adjacent to the vascular bundle is composed of rectangular cells whose contents stain very deeply with safranin. Next is a layer containing nuclei but very little cytoplasm. The distal layer has polyhedral cells with contents similar to those of the basal layer.

Pseudogamous Blackberries

Only one variety of pseudogamous blackberries, namely Evergreen (*R. laciniatus*), was studied histologically. The gross development of the ovary and ovule is similar to that of Eldorado. Nevertheless some anomalies were found. Two ovaries each contained three ovules but the upper two had degenerated. Six drupelets possessed twin seeds in both of which endosperm had been formed.

The chief difference noted between the syngamic and pseudogamic species was in the establishment of the megagametophyte. The growth of the individual gametophyte follows the normal pattern (19) from the megaspore stage to maturity. Usually only a single gametophyte develops in the syngamic species, whereas several are formed in the pseudogamic ones. Of the 75 Evergreen ovules examined between the bud stage when most of the gametophytes contained four to eight nuclei and two days after pollination,

34.7% contained two, 10.7% had three, and 2.7% had four gametophytes. The multiple gametophytes were not always at the same stage of development (Fig. 13). Frequently one would have eight nuclei and its twin four nuclei; or one would be disintegrating and the other in a robust condition. Two ovules contained gametophytes growing end to end but separated by a few undifferentiated cells. The supernumerary gametophytes disintegrate as development progresses. Similar observations were reported by Christen (2). No multiples were observed later than seven days after pollination.

Besides the variability in number of gametophytes there was considerable other irregularity. Three megagametophytes were observed in which the egg apparatus was at the chalazal end. The megagametophytes of four ovules extended down into the vascular bundle. One of these had grown through the chalazal end of the ovule and formed a vesicle in the ovarian cavity. In pollinated material, seven gametophytes were seen which had three apparently normal polar nuclei in contact and one which had four. One gametophyte had two eggs and another lacked an egg altogether. The largest cell of the egg apparatus was a synergid in one instance. In still another the egg and synergids formed a linear row of cells. Two gametophytes were observed with seven nuclei clumped together. This group was located at the micropylar end in one case and at the chalazal end in the other.

Development of the embryo and endosperm is, in general, similar to that in the syngamic blackberries studied. Cell-wall formation is not dependent on the embryo since at eight to nine days after pollination the largest embryos, 96- and 98-celled, were in free-nucleate endosperms, and two of the endosperms associated with disintegrating eggs were cellular at the micropylar end.

The mature embryo occupies almost the entire seed. The cotyledons are usually three or four times as long as the hypocotyl. There is no plumule; the epicotyl is only a slight hump between the cotyledons. Vascular strands extend the entire length of the cotyledons, branching several times just before reaching the epidermis. Nutrients appear to enter the embryo chiefly through the tips of the cotyledons; the endosperm between the embryo and the chalazal region does not contain any reserve food at maturity (Fig. 10). A thin layer of collapsed endosperm cells lies between the embryo and the turgid endosperm. The contents of these cells have been resorbed, and in many instances the walls have been reduced to a homogeneous material. The demarcation between disintegrated and plump endosperm is clear-cut. Contrary to Péchoutre's findings (21), the layer of endosperm surrounding the embryo varies considerably in thickness depending on the individual seed and the location within the seed. The mean thickness of this layer of endosperm is approximately two cells. However four or five layers of cells are usually present along the flat side of the cotyledons and frequently all the endosperm cells at the base of the cotyledons have been resorbed so that the embryo is in contact with the seed coat.

The shape and size of the mature embryos varied considerably. This was usually correlated with the dimensions of the seed. Three abnormal embryos

were observed however. One had a hypocotyl almost as long as the cotyledons. Both the embryo and endosperm were deficient in food reserves. The second abnormal embryo had one cotyledon at least five times as large as the other one. The epicotyl and hypocotyl appeared normal, but there was a deficiency of starch in the endosperm. A goodly volume of the endosperm towards the chalazal end of the seed contained no food reserves and the remainder had an abnormally small amount. The third of these embryos had only one cotyledon. This embryo possessed a plumule of two leaves. The starch content of both embryo and endosperm was normal. The lack of a cotyledon does not in itself prevent the seed from sprouting, since a BB-1 seedling with a single cotyledon was obtained.

The appearance of the reserve food bodies of the embryo and of the endosperm (Fig. 19) differed. Those of the embryo were round and clear-cut, staining intensely with safranin, Delafield's haematoxylin, or iodine. Those of the endosperm were about the same shape, but were somewhat larger and did not stain as intensely; they contained regions of various sizes which did not take up the dye. Nuclei in the mature seeds were not always easily recognized since a layer of deeply-staining material was usually found around each one. The nucleoli could usually be identified, especially when stained with safranin.

A similar study of the development of the seed following the cross, Evergreen × BB-1, was made but no significant differences were found in the early stages. The "hybrids" had the largest embryos and, at the 15-day interval, averaged a much greater endosperm development than Evergreen selfed. Nutrition in many embryos of Evergreen selfed seemed to be by an extraction of the protoplasm of the adjacent endosperm cells, whereas in the hybrid the whole cells were being disintegrated. This region of disintegration extended in the most advanced seeds through the center of the endosperm about halfway to the chalazal end. Evidence from earlier stages indicated that this extensive disintegration was probably a result of the greater development of the embryo rather than a characteristic of the "hybrid".

Development of Abortive Seed

A high percentage of aborted seeds was found in the gross examination of the mature nutlets. Since this aspect of the problem had not been considered at the time collections were being made, very little material was obtained between 15 and 60 days after pollination. However, earlier and later material, sufficient to determine the course of development, was obtained.

As was mentioned earlier, the primary endosperm nucleus usually divided two days after pollination and the egg one or two days later. The seed was approaching its mature size by the sixth day (cf. Figs. 18 and 26). Practically all of the enlarged seeds contained active endosperms, but many of them lacked embryos. The numbers of seeds with and without a developing embryo which were obtained at different ages following certain pollinations are given in Table IV.

TABLE IV

NUMBER OF DEVELOPING EMBRYOS IN DEVELOPING SEEDS AT DIFFERENT AGES

Cross	Age (days)	Embryo formed			Embryo not formed					
			Endosperm nuclei		Healthy egg			Degenerated egg		
		No. of seeds	Av.	Range	No. of seeds	Endosperm nuclei		No. of seeds	Endosperm nuclei	
						Av.	Range		Av.	Range
BB-1 × self	6	15	132*	65– 238	2	127	76– 178	1	114	—
	8	6	138	111– 214	5	127	112– 136	1	126	—
	10	3	146*	117– 200	2	157	124– 189	0	—	—
	15	10	1204	509–2663	0	—	—	1	533	—
		34			9			3		
Evergreen × self	5	1	124	—	7	28	15– 46	1	24	—
	6	7	69	32– 131	4	44	16– 66	1	32	—
	8	7	236	190– 305	1	122	—	3	361	227– 478
	10	7	356*	239– 517	3	724	475–1093	1	1715	—
	15	5	1507	750–3271	0	—	—	0	—	—
		27			15			6		
Evergreen × BB-1	5	1	67	—	2	51	50– 52	0	—	—
	10	2	291	162– 419	1	337	—	3	399	113– 750
	15	5	5438	3275–8605	0	—	—	2	4468	3952–4984
		8			3			5		

** Does not include one seed in which the endosperm was degenerating.*

The majority of developing seeds lacked a proembryo at five days. Possibly a few of these "eggs" would have undergone division and formed an embryo. One zygote was observed in prophase five days after pollination (Fig. 11), and there was also some variation in the size of the embryos at later stages.

Twelve BB-1 seeds, eight days after pollination, were critically studied. One of these had an 18-celled embryo with an abnormally large basal cell and a 214-nucleate endosperm. The number of endosperm nuclei in the remaining seeds varied from 111 to 136. Five of these seeds had healthy embryos ranging in size from 21 to 49 cells (Fig. 14). The other six seeds all had undivided "eggs", either degenerated (Fig. 15) or apparently healthy (Figs. 16 and 17). The associated synergids were in various stages of degeneration. The seeds from which Figs. 14 to 17 were drawn all came from a single BB-1 fruit.

Two seeds with unusual contents were found in 10-day material. One had a four-celled embryo and 200-nucleate endosperm. This was the only example of a healthy BB-1 embryo which had less than 10 cells later than five days after pollination. The other abnormal seed contained a disintegrating 42-celled embryo and only one identifiable nucleus in the endosperm. There were a few other concentrations of protoplasm in the endosperm, but whether they represented disintegrated nuclei could not be ascertained. The chalazal end of this seed had not differentiated except that the walls in the lowest region were more rigid than the rest. The cells in the location of the cytoplasmic region had only a little disintegrated protoplasm. The space usually occupied by the endosperm was partially filled by nucellar cells containing little or no protoplasm. A few cells with homogeneous brown contents were scattered through the nucellus. The seed was showing general signs of degeneration and collapse.

FIG. 8. Chalazal portion of the BB-1 ovule in Fig. 7. A conductive strand passes from the vascular bundle to the megagametophyte (× 225).

FIG. 9. Chalazal portion of a selfed BB-1 seed 15 days after pollination showing vacuolate, cytoplasmic, and disintegrating regions between the vascular bundle and endosperm (× 225).

FIG. 10. Chalazal portion of a mature open-pollinated Evergreen seed. The vacuolate region has divided into three layers, the cytoplasmic region has disappeared, and the innermost region is reduced to a layer of cell walls. The endosperm at the tip of the cotyledons contains no ergastic materials (× 150).

FIG. 11. Egg apparatus and endosperm of a five-day selfed BB-1 seed. The nuclei of both male and female gametes in the zygote are in late prophase (× 525).

FIG. 12. Twin embryos in a BB-1 seed, six days after pollination (× 525).

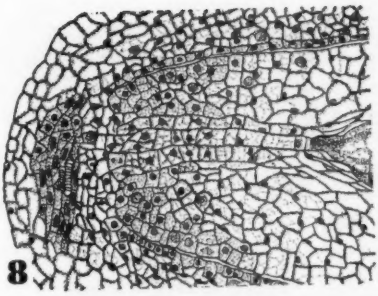
FIG. 13. Three gametophytes in an Evergreen ovule. These are at different stages of development (× 375).

FIG. 14. Micropylar concentration of endosperm nuclei in an eight-day BB-1 seed containing a vigorous 49-celled embryo (× 525).

FIG. 15. Degenerating "egg" and degenerating synergids with associated endosperm in an eight-day BB-1 seed (× 525).

FIG. 16. Healthy "egg" and degenerating synergids with associated endosperm in an eight-day BB-1 seed (× 525).

FIG. 17. Similar to Fig. 16. One synergid is degenerating and the other showing the first signs of disintegration (× 525).



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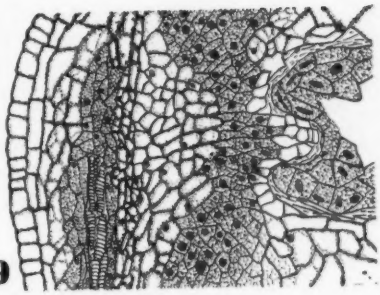

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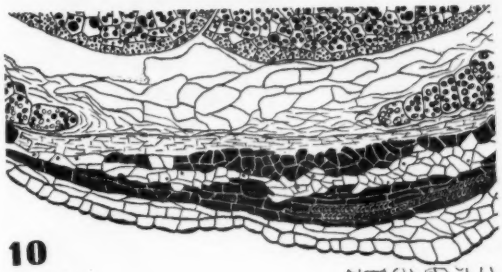

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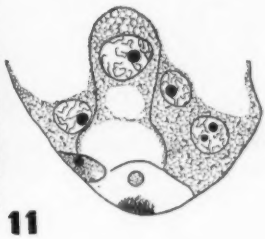

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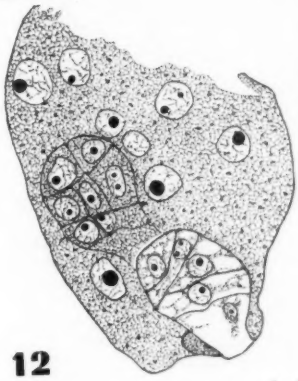

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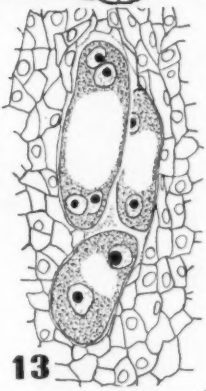

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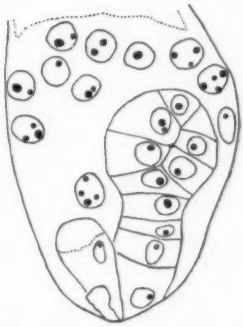

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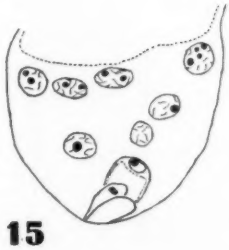

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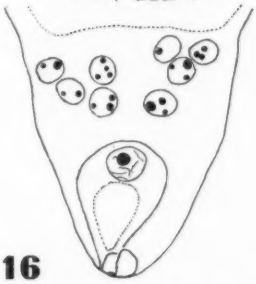

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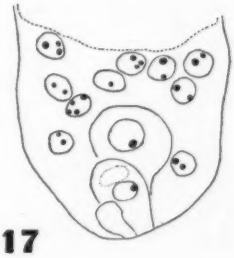

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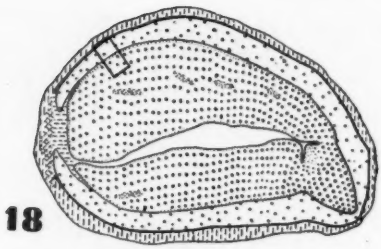

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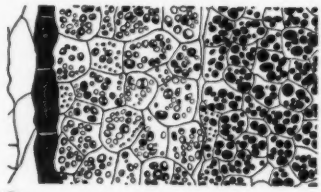

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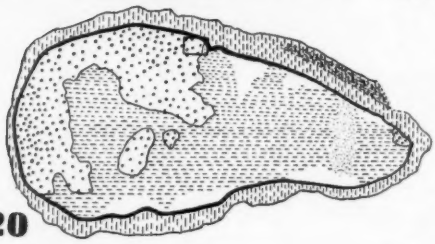

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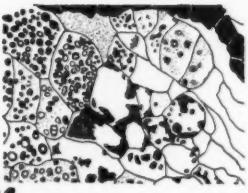

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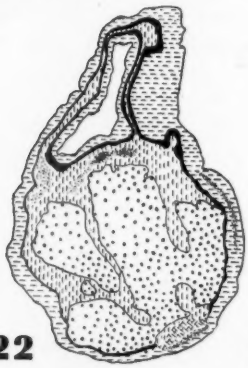

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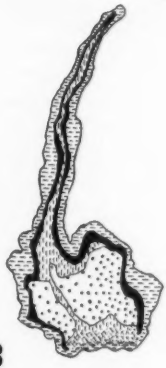

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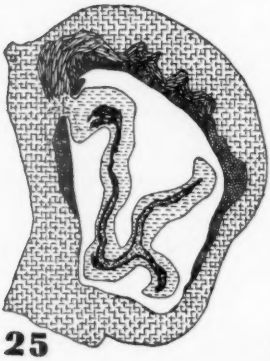

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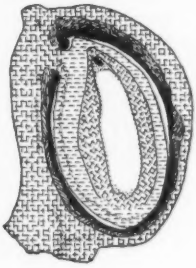

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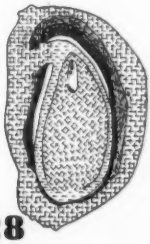

28

Eleven BB-1 seeds collected 15 days after pollination were examined. Nine of these contained healthy embryos with cotyledons beginning to differentiate. The number of nuclei in the accompanying endosperms ranged from 672 to 2663. The seeds with the larger endosperms also contained the more highly differentiated embryos. One seed contained an endosperm with 509 nuclei. The embryo proper, although rather small, appeared healthy, but the cells of the suspensor were very deficient in cytoplasm. The other seed contained an active 533-nucleate endosperm but no embryo. The "egg" was beginning to disintegrate.

Division of the "egg" in Evergreen selfed did not take place as early as it did in BB-1, but only two of the 19 embryos examined in seeds eight days or more after pollination had less than 39 cells. These had 4 and 15 cells respectively. The endosperm appeared normal in every instance. The other eight seeds which were examined at these ages also contained abundant active endosperm. The egg or zygote in four of them appeared healthy and capable of division, whereas in the others it was clearly disintegrating.

One seed of Evergreen × BB-1 collected 15 days after pollination contained noteworthy contents. The cells of the egg apparatus were still recognizable although their nuclei had disintegrated. The endosperm at the micropylar end was composed of very large, highly vacuolate cells containing small nuclei. It was multinucleate and mitotically active toward the chalazal end.

FIG. 18. Longitudinal section through a plump, mature Evergreen seed. It contains a normal embryo. The embryo is surrounded by a layer of endosperm which lacks ergastic contents at the chalazal end of the seed (× 25).

FIG. 19. Enlargement of the area outlined in Fig. 18 to show the appearance of the ergastic materials (× 150).

FIG. 20. Longitudinal section through a mature Evergreen seed that showed only slight shrinkage. There is some starch storage at the micropylar end but no evidence of an embryo. The section is not quite median (× 25).

FIG. 21. Enlargement of the area outlined in Fig. 20 to show the appearance of the ergastic materials (× 150).

FIG. 22. Longitudinal section through a partially collapsed, mature Evergreen seed. The chalazal half of the seed is distended with endosperm cells most of which contain starch. The micropylar end is collapsed but contains endosperm cells which have lost their contents. Strands of collapsed endosperm cells extend to the chalazal end. The micropylar end is not solid because the seed was wrinkled (× 25).

FIG. 23. Similar to Fig. 22, but there has been less starch storage (× 25).

FIG. 24. Longitudinal section through a mature Evergreen seed which appeared to be completely collapsed. Again no embryo is present. A very small amount of starch is stored at the chalazal end of the endosperm (× 25).

FIG. 25. Mature, poorly-developed Evergreen drupelet. The two layers of the endocarp show some differentiation at the distal end. The ovule has collapsed and is greatly wrinkled (× 18).

FIG. 26. Well-developed BB-1 drupelet, six days after selfing. The endosperm is 165-nucleate, the embryo 31-celled, and the endocarp has differentiated all around the drupelet (× 18).

FIG. 27. Undeveloped BB-1 ovary, six days after selfing. The ovule has collapsed and the endocarp has not differentiated (× 18).

FIG. 28. Poorly-developed BB-1 drupelet, six days after selfing. The polar nuclei are undivided, the egg has divided once, and the endocarp has differentiated only at the micropylar end (× 18).

Numerous round, deeply staining bodies of unknown composition or origin were present at the chalazal end. (These bodies were also found in many other endosperms but no correlation could be established either with the type of pollination or with the degree of maturity.) The maternal tissue of the chalazal region contained more cells than usual and they were in active division.

Some of the seeds with apparently normal endosperm would never possess an embryo. In 17% of the seeds without embryos at five to six days after pollination, the three cells of the egg apparatus all showed signs of disintegration. In the 8- to 15-day material 48% of these seeds had disintegrating eggs.

At 5 to 15 days 26% of the BB-1 seeds and 39% of the Evergreen seeds were developing without embryos (Table IV). This compares with 28% and 51% defective seeds in the mature nutlets of these varieties (Table III).

When these shrunken seeds were examined macroscopically at maturity, the degree of development appeared to vary continuously from no contents to normal contents. Many of the seeds had collapsed completely, many others were plump only at the chalazal end, some had collapsed only at the micropylar end, and a few were shrunken in the middle. Microscopic examination of 62 of these seeds showed a slightly different condition (Figs. 20 to 24). Although the illustrations are from the Evergreen material, the histological picture was similar in the several blackberry varieties examined.

Some of the seeds which were shrunken only in the center contained embryos but only one of these had an embryo that appeared viable. Large embryos with very little reserve starch were found in eight seeds. Two others contained dead embryos about one-quarter normal size. One seed contained an embryo that was normal in shape but the cells of the cotyledons had very little cytoplasm and those of the hypocotyl contained considerable granular material. The rest of the shrunken seeds showed no evidence of an egg or embryo although some had a few starch-filled cells at the micropylar end (Fig. 20).

The endosperm in the aborted seeds was peculiar. Frequently the chalazal endosperm cells were of normal proportions and contained the customary protoplasmic and ergastic materials. Clumps of this tissue were usually separated by strands of collapsed cells which contained no protoplasm—only the cell walls remained. The proportions of distended and collapsed cells varied greatly from seed to seed. Usually these regions of collapsed tissue expanded, more or less abruptly, until they merged and included all the cells at the micropylar end. The contents of the protoplasmic cells differed from cell to cell (Fig. 21). Some cells contained very dense starch grains similar to those of the embryo (Fig. 19). Other cells contained starch grains which resembled those of the endosperm in a viable seed. The contents of some cells were in granules which showed disintegration to a greater or less extent; the contents of other cells or groups of cells were completely disorganized.

On macroscopic examination, many of the aborted seeds appeared to be completely collapsed. On microscopic examination such seeds were found to contain a few somewhat shrunken endosperm cells containing a little ergastic material at the chalazal end (Fig. 24). All of the seeds obtained from nutlets which appeared normal showed evidence of endosperm development.

Development of Abortive Drupelets

Some drupelets are listed in Table II as being poorly developed. These, at maturity (Fig. 25), were $\frac{1}{8}$ to $\frac{1}{3}$ the size of the well-developed drupelets. The exocarps were small but normal; the endocarps were of variable thickness but never fully formed. Thickening of the endocarp took place first at the stylar end and usually did not proceed as far as the base. The ovules had enlarged somewhat, but, by maturity, had completely collapsed and showed no evidence of either embryo or endosperm. The cells of the integuments were dead.

Some of these aberrant drupelets were examined at 5 to 10 days after pollination, i.e., near the time when they could first be identified. By 10 days the ovules had collapsed, the protoplasm of the nucellus had disappeared, and the egg apparatus had disintegrated to a dark mass. No evidence of endosperm was seen in the ovules examined. A somewhat similar condition was evident in eight-day material; there was no definite evidence for division of either the egg or the endosperm. In all of these drupelets, the ovule and its contents showed signs of degeneration and were beginning to collapse.

The drupelets which are classified as poorly developed five and six days after pollination were a heterogeneous group. A number contained embryos and endosperms which were small for this age but were apparently healthy. Others had developing endosperms, and "eggs" which were either sound or showed signs of deterioration. One seed (Fig. 28) contained a two-celled proembryo and undivided polar nuclei. In still others, neither the egg nor the endosperm mother-nucleus had divided. The ovules in some of these poorly-developed drupelets had collapsed a little, but others were filled out to approximately the shape and size of seeds containing active embryos and endosperms. Well-developed, undeveloped, and poorly-developed drupelets from a single BB-1 fruit, six days after pollination, are shown in Figs. 26, 27, and 28.

Discussion

Blackberry ovaries which do not contain ovules, or those in which both the ovules have degenerated, do not show any enlargement. The same is true of the great majority of the unpollinated ones. The drupelets which have been recorded as poorly developed enlarge during the first week, almost as rapidly as those which are going to produce normal seeds, but development of the endocarp and ovule ceases some time during the first period of rapid expansion. The exocarp remains alive during the second period of slow growth and takes part in the final rapid expansion as the fruit ripens. The final enlargement must be conditioned by the hormonal system of the whole aggregate fruit, since these small drupelets ripen at the same time as the larger ones.

Poorly-developed fruits known as "buttons" are frequently produced by the J. H. Hale peach. Dorsey (10) has suggested that these are caused by single fertilization. This is probably not the cause of the poorly-developed drupelets in blackberries as the primary endosperm nucleus does not divide. Natural parthenocarpy such as reported in maize by Britten (1) is a more likely explanation.

About 50% of the ovaries showed no development. This lack of enlargement may be associated with failure of pollination, faulty development of the ovule or gametophyte, or with some form of gametic incompatibility. It is believed that the last-suggested cause operates in the majority of cases because abundant pollen was usually applied, pollination at more than one time did not increase the drupelet set, and irregularities of the ovary and ovule were rarely seen.

In the drupelets which appear normal but which contain aborted seeds, the pollen tube penetrates the gametophyte, and the endosperm mother-nucleus usually, if not always, divides. The "egg" fails to develop into an embryo in the majority of these seeds. A few seeds were found in which both embryo and endosperm were formed but one or the other (usually the embryo) was unable to develop normally. Hence incompatibility is expressed to a greater extent when the male and female genomes are in the ratio 1 : 1 than when they are in the ratio 1 : 2. The double portion of maternal genomes seems to outweigh the adverse effects of the single portion of paternal genomes.

The chief reason for the formation of so many imperfectly developed seeds is believed to be aneuploid chromosome numbers, although genic self- and cross-incompatibility might be a minor factor (22). This hypothesis is supported by similar data for sweet cherries which are aneuploids (3) and triploid apples (23). Crane and Lawrence (7) found some cherry seeds which were plump and apparently capable of germinating, some which showed various degrees of degeneration, and some which possessed only the testas. Crane and Brown (5) concluded that the low degree of embryo development and, in extreme cases, the absence of seeds after certain crosses in plums resulted from the "discordance of the parental chromosome distributions". Practically all backberries show considerable meiotic irregularity and pollen sterility, so one would expect to find many aneuploids. Since only a very few aneuploid species and varieties have been reported (11, 13, 15), and since all the well-developed seeds will germinate if given favorable conditions, it is probable that many of the aneuploids are eliminated as the imperfectly developed seeds.

The data in Table IV show that seeds having a normal endosperm-content frequently will reach a high degree of development without the egg (or zygote) undergoing mitosis. The egg was in an advanced stage of degeneration in approximately one-third of such seeds. No suggestion of nucellar embryony was found in the seeds examined.

The results of this study offer a partial explanation for the low germination of blackberry "seed" obtained by many investigators. Nutlets from some of

the classes of drupelets would seldom be planted since they float on water and would be discarded with the exocarps at harvest time. Some of the lightweight but normal-appearing nutlets undoubtedly have been planted by some investigators. These and the heavy nutlets which contain no embryos, or aborted ones, will not germinate.

The germination of blackberry seed which appears normal can be increased by favorable environmental conditions. If the endocarps are removed a low percentage of blackberry seeds kept at room temperature will germinate whereas a high percentage of raspberry seeds will germinate (18). The polyploidy of the blackberry or, possibly, a postulated aneuploidy of many seeds may account for this difference in germination. Full germination of viable blackberry seeds can be obtained by removal of the endocarps followed by low temperature treatment.

The interrelationships of the embryo, endosperm, and maternal tissues are similar in the pseudogamous and syngamous species of *Rubus* which were examined. No marked differences were obtained after various pollinations, even when another species was used as the pollen parent. However, some information concerning the role and interdependence of these tissues was obtained from a study of the aborted seeds.

The endosperm derives its nourishment from the more mature cells of the nucellus, at least partially, by digesting those in immediate contact with it. The great mitotic activity in the chalazal region of the nucellus after pollination indicates that the digestive enzymes produced by the endosperm do not have a deterrent effect on mitosis. The lack of stored food in young seeds in which the endosperm either did not divide or aborted at an early stage is evidence that the hormones produced by this tissue stimulate the transfer of nutrients to the seed. The chalazal cells are filled with dense cytoplasm at the mature-gametophyte stage, but there is no accumulation of ergastic material until the endosperm is well formed.

The embryo obtains its nourishment from the endosperm. There is no evidence that any of the endosperm nuclei are digested during the multinucleate stage of its development. The embryo's nutriment comes directly from the cytoplasm of the endosperm with which it is in contact. When the micropylar end of the endosperm becomes cellular, digestion of these cells takes place most rapidly at the cotyledonary end of the embryo. The endosperm is not completely resorbed at the sides of the embryo during the early stages of development. These lateral cells remain mitotically active for some time.

The endosperm develops independently of the embryo until at least 15 days after pollination. This is indicated by its behavior in seeds lacking embryos. The number of endosperm nuclei at a given age during this period of growth is approximately the same whether an embryo is present or not. During this stage a little protoplasm is removed from the micropylar end of the endosperm by the developing embryo.

In later stages of development the embryo stimulates the movement of nutrients to the seed and influences their distribution. In the absence of an embryo, ergastic materials are deposited almost exclusively in the chalazal end of the endosperm. Mature seeds which lack embryos usually contain a much smaller quantity of storage products than those with embryos.

Acknowledgments

The writer gratefully acknowledges his indebtedness to Prof. R. A. Brink for counsel in the genetical aspects of the work and to Prof. D. C. Cooper for supervision of the histological phases of the study.

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QUANTITATIVE STUDIES ON THE SURVIVAL OF FUNGI IN FIVE-YEAR-OLD DRIED SOIL CULTURES[1]

By R. G. Atkinson[2]

Abstract

Of 51 dried soil cultures of fungi isolated from soil, only 12, comprising seven of the 32 genera represented, were viable after five years. Platings, directly from the cultures, of 5-, 50-, and 500-mgm. samples with oxgall-amended potato dextrose agar revealed extreme variations in viability of surviving fungi. Population levels were so high in cultures of *Rhizopus nigricans*, *Aspergillus fumigatus*, *A. sydowi*, and *A. ustus* that plate counts were not obtained, even when a 5-mgm. sample was apportioned among five plates. Cultures of *Chaetomium indicum* and of *C. spirale* contained an estimated population of approximately one and one half million, and 450,000 viable centers per gram, respectively, the highest counts obtained. The next most viable cultures were those of *Fusarium oxysporum* var. *redolens* and of *Circinella spinosa*, but these contained only about 60,000 and 20,000 centers per gram, respectively. Next came *Penicillium janthinellum* with an estimated 4000 centers per gram. The least viable cultures were those of *Penicillium purpurogenum* and of two isolates of *F. oxysporum* whose population level was down to about 100 to 200 viable centers per gram.

Introduction

The relatively few references in the literature to the storage of fungi in dried soil cultures have already been reviewed in a previous paper (1). Bakerspigel (2) has recently reported on the survival of fungi, mostly dermatophytic and systemic pathogens of animals, in loam, clay, sawdust, peat, sand, and kieselguhr. The most suitable of these materials for storage proved to be loam, in which *Phoma* sp. and *Fusarium* sp. were still viable after 48 months and *Hormodendrum* sp. *b* and *Phyllosticta* sp. after 32 months. On the other hand, a loam culture of *Hormodendrum* sp. *a* was not viable when tested after only five months. All of the loam cultures of the dermatophytes and systemic fungi were still viable after 13 months.

The results presented here were obtained from tests made on a wide range of soil fungi that were isolated during microbiological studies on potato scab (9), and for most of them no information is available regarding their capacity to survive lengthy storage in dry soil.

Materials and Methods

The fungi under investigation had been isolated from soil samples taken at Ancaster, Ontario, and cultured for periods ranging from a few months to slightly more than a year on soil-extract agar slants. Subsequently, transfers

[1] *Manuscript received June 14, 1954.*
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of the following fungi* were made to a thoroughly moistened and sterilized composted loam plus sand mixture in ordinary culture tubes, 18 × 150 mm.

Phycomycetes

2 *Actinomucor repens* Schost.
10 *Circinella spinosa* Tiegh. & Le M.
41 *Rhizopus nigricans* Ehr.

Ascomycetes

59 *Chaetomium indicum* Corda
60 *Chaetomium spirale* Zopf

Fungi Imperfecti

45 *Acrostalagmus albus* Preuss var. *varius* Jensen
47 *Alternaria tenuis* auct. sensu Wiltsh.
1 *Aspergillus fumigatus* Fres.
50 *Aspergillus sydowi* (Bain. & Sart.) Thom & Church
55 *Aspergillus ustus* (Bain.) Thom & Church
5 *Bispora* sp. Corda
42 *Botrytis cinerea* Pers.
4 *Botrytis terrestris* Jensen
16 *Cephalosporium acremonium* Corda
19 *Cephalosporium* sp. Corda
7 *Chaetomella horrida* Dud.
6 *Cladosporium herbarum* (Pers.) Link
8 *Coniothyrium fuckelii* Sacc.
12, 54 *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen
13 *Fusarium merismoides* Corda
57 *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen var. *redolens* (Wr.) Gordon
14 *Geotrichum candidum* Link
52 *Gliocladium atrum* Gilman & Abbott
56 *Gliocladium roseum* (Link) Thom
44, 51 *Gliomastix convoluta* (Harz) Mason var. *felina* (Marchal) Mason
61 *Gonytrichum caesium* Nees ex Wallr.
40 *Haplaria ? grisea* Link
29 *Haplographium chlorocephalum* (Fres.) Grove
20 *Heterosporium terrestre* Atk.
21 *Hormodendrum cladosporioides* (Fres.) Sacc.
22 *Hormodendrum hordei* Bruhne
18 *Hyalopus ater* Corda
27 *Myrotheciella* sp. Speg.
24 *Myrothecium roridum* Tode ex Fr.
25 *Myrothecium verrucaria* Ditmar ex Fr.
28 *Oospora sulphurea* (Preuss) Sacc. & Vogl.
53 *Papularia sphaerosperma* (Pers.) Höhnel
32 *Papulaspora* sp. Preuss
31 *Penicillium canescens* Sopp
39 *Penicillium cyclopium* Westl.
46 *Penicillium herquei* Bain. & Sart.
15 *Penicillium janthinellum* Biourge
48 *Penicillium purpurogenum* Stoll
9, 11 *Stilbella bulbicola* P. Henn.
36 *Trichoderma album* Preuss
35 *Trichoderma koningi* Oud.
34 *Trichoderma lignorum* (Tode) Harz
38 *Volutella roseola* Cooke

Soil cultures were prepared on Dec. 15, 16, and 29, 1948 with the exception of that of *Heterosporium terrestre*, which was set up on Mar. 4, 1949. The cultures dried out within a few weeks and during the five-year interval they were stored in an enclosed bookcase in diffuse light, at room temperature.

* *These fungi are also maintained on potato dextrose agar slants and are listed, with culture numbers and pertinent information, in the Directory and Catalogue of Collections of Micro-organisms maintained in Canada, National Research Council, Ottawa, Ontario.*

In order to obtain quantitative data concerning the residual population level in surviving cultures, the soil plate method of Warcup (11) was adopted in which 5- to 15-mgm. samples of field soil are placed in each plate before introducing the medium. In the soil cultures, however, the number of viable centers of any fungus surviving the stringent storage conditions was expected to be so low that larger samples would be necessary if suitable plate counts were to be obtained. Some cultures confirmed this prediction; others did not.

On the basis of preliminary platings to determine the required range of sample size, 5, 50, and 500 mgm. were removed from the upper inch of soil in the tubes after the soil had been thoroughly pulverized. Weighing was carried out on an alcohol-flamed watch glass on which a film of residual soil always remained. The plates were poured with potato dextrose agar supplemented with oxgall* (6, 8) at the rate of 2% and incubated at 27° C. for a period of from four to seven days. Colonies were counted under a bacteriological counter and, where they were extremely small or crowded, under a dissecting microscope having a maximum magnification of ×60.

Soil cultures that proved to be viable in the first test were replated in duplicate, with the following changes in procedure. Certain cultures, from which even 5-mgm. samples gave rise to excessive plate growth, were retested by distributing this amount to three or five plates, as deemed necessary. Other viable cultures were retested by distributing 50-mgm. or 500-mgm. samples to three or four plates in the hope of obtaining suitable counts.

Another change in procedure was adopted in the second test because it was felt that the unplated residual film of soil on the watch glass introduced a serious error in the plate counts obtained, especially from those cultures that contained exceedingly high numbers of viable centers. Accordingly, soil samples were weighed on flamed microscope cover glasses from which approximately equal portions were carefully distributed to all but the last plate in a series. This plate was reserved for the cover glass and its thin film of adherent soil.

There appeared to be a remote possibility that the fungi in some of the nonviable cultures may have been so sensitive to the inhibitive action of oxgall that the long dormant cells, although viable, failed to germinate. Therefore, approximately 500 mgm., on a rough volume basis, of each of the negative cultures was replated, in duplicate, on unsupplemented potato dextrose agar.

Results and Discussion

Of the 51 cultures tested, comprising 32 genera of soil fungi, only 12, representing seven genera, survived the five-year period in dry soil. The numbers of viable centers remaining in these cultures, as determined by plate counts, are presented in Table I. This table also shows that the amount of viable inoculum in the soil film remaining on the cover glasses used for weighing was exceedingly high in some cases, and emphasizes the necessity of including this residue if accurate plate counts are desired.

* *Bacto-Oxgall prepared by Difco Laboratories.*

TABLE I

NUMBER OF VIABLE CENTERS IN SAMPLES PLATED DIRECTLY FROM FIVE-YEAR-OLD DRY SOIL CULTURES

Fungus*	Test	Wt. of sample, mgm.	Replicate	Plates per sample	No. of colonies			
					Residue on cover glass	Total in sample	Estimate for 1 gm.	
							Replicate	Av.
Circinella spinosa		5	1	1	—	73	—	14,600
	1	50	1	1	—	551	—	11,020
		500	1	1	—	?§	—	—
	2†	50	1	3	209	1178	23,560	21,440
			2	3	102	966	19,320	
Chaetomium indicum	2	5	1	5	1524+‡	5852+	1,170,400+	
			2	5	1364+	5698+	1,139,600+	1,152,000+
Chaetomium spirale			1	5	557+	2334+	466,800+	
	2	5	2	5	523+	2263+	452,600+	459,700+
Penicillium janthinellum		5	1	1	—	21	—	4200
	1	50	1	1	—	194	—	3880
		500	1	1	—	953+	—	1906+
	2	500	1	4	243	2098	4196	4431
			2	4	357	2333	4666	
Penicillium purpurogenum		5	1	1	—	0	—	—
	1	50	1	1	—	6	—	120
		500	1	1	—	22	—	44
	2	500	1	3	6	102	204	180
			2	3	5	78	156	
Fusarium oxysporum (No. 12)		5	1	1	—	3	—	600
	1	50	1	1	—	11	—	220
		500	1	1	—	53	—	106
	2	500	1	3	0	11	22	31
			2	3	0	20	40	
Fusarium oxysporum (No. 54)		5	1	1	—	2	—	400
	1	50	1	1	—	1	—	20
		500	1	1	—	59	—	118
	2	500	1	3	0	40	80	153
			2	3	0	113	226	
Fusarium oxysporum var. *redolens*		5	1	1	—	239	—	47,800
	1	50	1	1	—	?	—	—
		500	1	1	—	?	—	—
	2	5	1	3	19	258	51,600	57,500
			2	3	44	317	63,400	

* *So many viable centers were present in the cultures of Rhizopus nigricans, Aspergillus fumigatus, A. sydowi, and A. ustus that plates were too crowded for counting, even when a 5 mgm.-sample was divided among five plates.*

† *In the second test, each sample was apportioned to several plates in order to obtain suitable distribution of colonies.*

‡ *The plus sign indicates an approximate count only, because of crowded colonies.*

§ *Too many colonies for counting.*

Of the three phycomycetes studied, only *Actinomucor repens* failed to survive the five-year storage in dry soil. The survival capacity of *Rhizopus nigricans*, however, was so great that even when only 5 mgm. of soil was distributed among five plates, and each plate therefore contained only about 1 mgm., so many colonies developed that growth was practically confluent across the entire surface. By comparison, much fewer viable centers remained in the culture of *Cincinella spinosa*, although its survival capacity could be rated as good. Although this fungus, like *Rhizopus* species, normally grows very rapidly, the presence of oxgall in the plating medium effectively curtailed the rate of spread so that the colonies remained more or less discrete as shown in Fig. 3. It is interesting to note that Fennell *et al.* (4) tested various methods of preserving cultures of mucoraceous fungi and found that, although lyophilization proved most effective, the soil culture technique ranked next. Because the soil culture method appeared to be an excellent means of preserving *Rhizopus nigricans* it seemed desirable to ascertain its performance under lyophilization. Dr. Dorothy Fennell* very kindly tested a lyophilized preparation (NRLL 1477) of this fungus and, in a recent communication, she states that this culture, set up on April 7, 1942, showed excellent viability when tested on Mar. 4, 1954, i.e. after a period of almost 12 years.

The two ascomycetes tested, *Chaetomium indicum* and *C. spirale*, showed a very high capacity for survival in dry soil and gave the highest plate counts that were obtained. The restrictive effect of oxgall on the spread of fungi is particularly well illustrated here, as shown in Figs. 1 and 2. Colonies of *C. spirale* were only 1 to 3 mm. in diameter, whereas those of *C. indicum* were even smaller, 0.25 to 1 mm. As a result, exceedingly high plate counts of these fungi were obtained.

With regard to the Fungi Imperfecti, very few of those tested were able to survive dry soil storage at room temperature for a five-year period. Of these, however, *Aspergillus fumigatus*, *A. sydowi*, and *A. ustus* appear exceptionally well adapted to withstand a desiccated soil environment. As in the case of *R. nigricans*, the plated samples from cultures of these fungi gave rise to almost confluent growth, even though each plate received only approximately one-fifth of the 5-mgm. sample. Again Dr. Fennell very kindly tested the viability of lyophilized preparations of the three *Aspergillus* species tested here in soil. The culture number and date of preparation of her cultures are as follows: *A. fumigatus* (NRRL 163), Apr. 15, 1942; *A. sydowi* (NRRL 250), Apr. 1, 1942; *A. ustus* (NRRL 278), June 23, 1942. As in the case of *Rhizopus nigricans*, these also showed excellent viability when tested on Mar. 4, 1954.

The capacity of *Fusarium oxysporum* var. *redolens*, shown in Fig. 4, to survive in dry soil proved to be very high, and in the group for which plate counts were available this fungus ranked third. This compares well with the survival of the muskmelon wilt *Fusarium* which Miller (7) and Bakerspigel (2) found to have remained viable in dry soil after test periods of three and four years,

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respectively. An extremely interesting effect of temperature on the survival of *Fusarium avenaceum* in dry soil cultures was noted by Cormack (3). He found that those stored at room temperature died out in the fourth year, whereas those kept at refrigeration temperature remained viable until the ninth year.

Penicillium janthinellum, shown in Fig. 5, ranked next in number of viable centers remaining but these viable centers numbered less than $\frac{1}{10}$ of those in the culture of *F. oxysporum* var. *redolens*.

Of the 12 surviving cultures, those of *Penicillium purpurogenum* and of the two isolates of *F. oxysporum* contained the least amount of viable inoculum. *P. purpurogenum* and *P. janthinellum* were the only ones of four species of *Penicillium* tested that survived and even the former species appeared close to extinction.

The nearly extinct condition of the culture of *F. oxysporum* in contrast to the excellent viability of the variety *redolens* of this species, as proposed by Gordon (5), is extremely interesting. This difference in survival lends strong support to Gordon's proposal "that *F. redolens*, which was included by Snyder & Hansen (10) in the synomomy of *F. oxysporum* Schlecht. emend. be differentiated from that species as a variety."

All of the cultures that proved nonviable when plated with potato dextrose agar supplemented with oxgall also failed to grow on the plain agar medium. Evidently the lack of growth from plated samples of the negative cultures was not due to an extreme sensitivity to oxgall, but to the absence of viable cells.

The present results indicate that tremendous differences exist in the ability of various fungi to maintain viable cells in dry soil at room temperature. Storage at refrigeration temperature, as Cormack (3) found to hold true for *Fusarium avenaceum*, probably would have enhanced the chances of survival of many of the cultures that had died out. Future work with the dry soil culture method could very profitably take this beneficial effect of low temperature storage into account.

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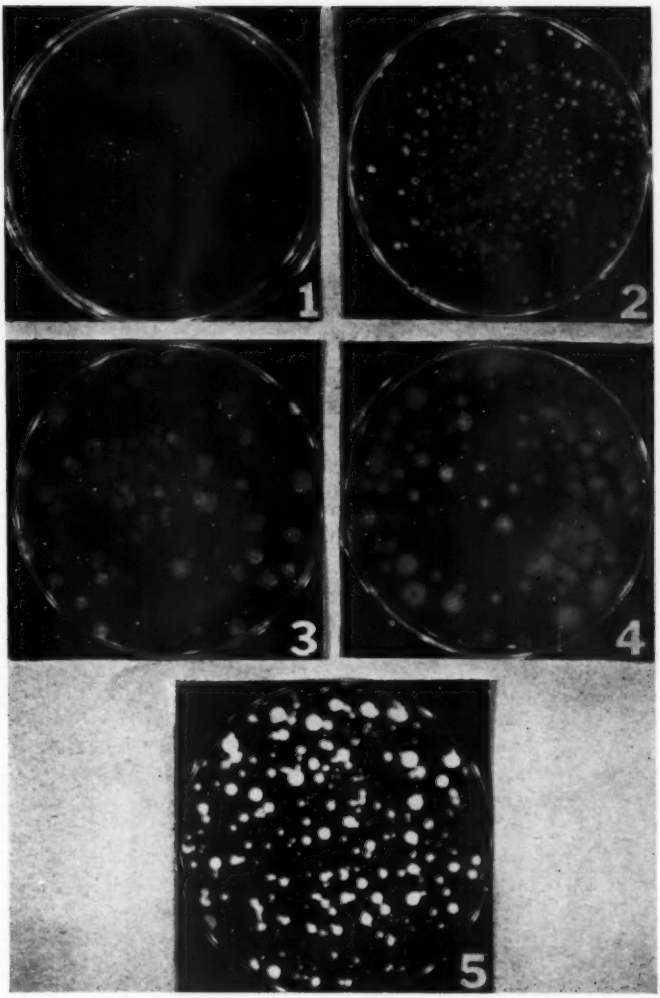


FIGS. 1–5. Inhibitive effect of oxgall-amended potato dextrose agar on fungous colonies developing from soil plated directly from dried soil cultures. All figures × $\frac{2}{3}$. FIG. 1. *Chaetomium indicum*. FIG. 2. *Chaetomium spirale*. FIG. 3. *Circinella spinosa*. FIG. 4. *Fusarium oxysporum* var. *redolens*. FIG. 5. *Penicillium janthinellum*.

INVESTIGATIONS OF *RHIZOCTONIA CROCORUM* (PERS.) DC. IN RELATION TO THE VIOLET ROOT ROT OF CARROT[1]

By N. J. Whitney[2]

Abstract

Violet root rot of carrot, caused by *Rhizoctonia crocorum* (Pers.) DC., appears in Ontario in midsummer, and by the fall progresses to cause deep lesions in the fleshy root of the carrot. Isolation of the fungus from diseased carrot was successful only from mature infection bodies. Of all the media tried meat–malt agar supported the best growth of *R. crocorum*. While three types of hyphae were produced only those of the subiculum propagated the fungus, and only the subiculum and sclerotia were capable of initiating a parasitic attack on carrots. *R. crocorum* can infect any part of a carrot root, does not require a wound infection court, and can spread uniformly over the carrot regardless of where primary infection occurs. Although the development of the carrot, the growth of *R. crocorum*, and the incidence of infection reached a maximum within the pH range of from 5.5 to 6.5, both host and fungus tolerate, and infection occurs within, a much wider pH range. The cardinal temperatures for infection were 5°, 20°, and 30° C., and there was a close correlation between the temperatures for best growth of the fungus and the host and that for maximum infection. Infection did not occur until the plants were eight weeks old, after which the disease spread until the carrots matured. Initial penetration occurred by hyphal tips forcing their way between the cells of the periderm. Both intercellular and intracellular hyphae were formed abundantly but they did not become established beyond the cambium. Infection bodies began in cells of the periderm where intracellular hyphae accumulated in masses prior to breaking through the outer layer of periderm cells. Two types of histological response to infection were observed: In one a cork layer, formed beneath the infection bodies, resulted in the development of shallow lesions, whereas in the other the infected tissues were broken down and deep lesions were formed in the carrot.

Introduction

Violet root rot, a disease found on carrots in the Thedford Marsh in Ontario, first threatened the carrot industry in that area in 1949 and was most serious in newly broken land which had been cropped for only one or two years. Besides carrots, various other vegetable crops of economic importance in the Thedford Marsh are known to be susceptible to this disease. For this reason and because the area concerned comprises some 6000 acres of arable muck, it was urgent that control measures applicable to the disease be developed, and yet few basic facts were known about the conditions under which the causal organism, *Rhizoctonia crocorum* (Pers.) DC., caused infection. To determine and evaluate some of these conditions experiments were designed concerning infection capabilities, temperature, and other requirements of the causal organism under controlled conditions in the greenhouse and under outside conditions in the field.

[1] *Manuscript received June 24, 1954.*

Joint contribution from the Department of Botany, University of Toronto, Toronto, Ontario, and (Contribution No. 1403) from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario. Based on a thesis presented in May, 1953, to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] *At the time, graduate student and lecturer, Department of Botany, University of Toronto; now Associate Plant Pathologist, Science Service Laboratory, Plant Pathology Section, Harrow, Ontario.*

Literature Review

Comprehensive reviews of the early literature on violet root rot are given by Duggar (4) and Garrett (7). The appearance of the disease and the relationship between host and parasite have been described by several authors including Prillieux (15) in 1879 and Faris (6) in 1921.

R. crocorum has a very wide host range including the roots of trees, shrubs, weeds, and a wide variety of vegetables, legumes, and other economically valuable plant species. In 1945 Hull and Wilson (11) reported that the known host range included 169 species distributed in 43 families. Recent work has shown that within this extended host range the fungus does not exhibit any narrow parasitic specialization. Thus Ogilvie *et al.* (12) found by experiment that an isolate of *R. crocorum* attacking asparagus could also attack carrot, sugar beet, mangold, parsnip, and potato, while Buddin and Wakefield (3) report cross-infections of the fungus from sugar beets to carrots and clovers, and from potato and mangold to various clovers. Violet root rot is world-wide in its distribution and has been reported at least once from Africa, Australia, and Asia, and many times from Europe and North America (4, 6, 9, 11, 14, 16).

Several authors, in describing outbreaks of violet root rot, have emphasized different soil, cultural, and climatic factors as affecting the disease. Van der Lek (17) found indications that manures rich in nitrogen favored the disease. In the opinion of Peyronel (13), the development of the fungus is favored by high water content of the soil, richness in organic material, and acidity. On the other hand, evidence is presented by Hull and Wilson (11) that severe infection occurs mainly in alkaline soil. Duggar (4), in his summary of early literature, points out that while several authors have emphasized the occurrence of the disease in wet situations, others have reported it in the driest situations permitting growth. Buddin and Wakefield considered that the high optimum temperature for growth of the fungus (25.5° C.) explained the geographical distribution of severe attacks in the field. According to Hull (10) the disease develops in crops on all types of soil, but mainly on light, gravelly, or peaty loams, though severe attacks sometimes occur in fields of clay loam.

Buddin and Wakefield (2) showed that *R. crocorum* will grow on a wide range of sterilized artificial material when in pure culture. Garrett (8), in his work on the effect of substratum on survival of the fungus colonies in the soil, was able to correlate the survival of the colonies with the production of firm resilient sclerotia around the center of the colony. Survival was increased by raising the carbohydrate concentration of the medium, but lessened by excess nitrogen. The depressing effect of excess nitrogen upon the production of sclerotia and survival of the colonies was attributed to an increased density of mycelial growth, which in turn led to reduction of carbohydrate level below that required for maturation of viable sclerotia.

The perfect stage of *R. crocorum* has been described by Buddin and Wakefield (3). They found fructifications of *Helicobasidium purpureum* Pat. in very close association with violet root rot in a number of localities and on three different hosts: red clover, dog's mercury (*Mercurialis perennis*), and nettle (*Urtica dioica*). Morphological characters of the mycelium of *H. purpureum* appeared identical with those of *Rhizoctonia crocorum* both on the host and in pure culture. In addition, typical violet root rot was produced by inoculation with mycelial cultures derived from three distinct basidiospore isolates of *H. purpureum*.

Observations on Violet Root Rot in Canada

Distribution and Field Studies

In Canada, as far as it is known, violet root rot is limited to a few isolated localities. In 1941 it was found in a plot of carrots at Comox, British Columbia. In 1952 a few specimens were located in a garden in central Saskatchewan. It has been reported at least once in Alberta and in Quebec and, recently, in the Thedford Marsh in Ontario. The disease is not confined to one particular type of soil since it was found in a sandy loam soil in Saskatchewan, in black muck in Ontario, and in clay loam in Quebec. In most of the reports carrot (*Daucus carota*) has been the host infected, but in the Thedford Marsh in Ontario, potatoes, celery, and goldenrod have also been found infected.

In the Thedford Marsh, violet root rot is most serious on newly broken land, but also occurs on land which has been under cultivation for as many as 20 years. This disease is not seed-borne and, since it occurs on newly broken land, the indications are that the causal organism, *R. crocorum*, is indigenous in the areas involved, particularly in the Thedford Marsh where numerous weed hosts comprise much of the natural cover.

In the fields under observation, both early and late carrots are grown, the former being harvested usually in mid-August and the latter in October. So far only the late carrots have been affected by the disease which appears late in the season attacking the carrots when their roots are well developed. Diseased carrots usually occur in patches throughout the field and, as symptoms do not show above ground, the affected areas are difficult to detect before harvest. At harvest the diseased carrots ordinarily are left lying in the field and are worked back into the soil thus building up the inoculum potential for the following crop. Besides carrots, many other vegetable crops of economic importance are grown in the Marsh, among them red beets, sugar beets, celery, potatoes, lettuce, cabbage, and onions, most of which are susceptible to violet root rot. Therefore, in almost every rotation, susceptible plants are provided for the continued survival of the parasite.

Description of the Disease on Carrots

Violet root rot first makes its appearance in midsummer, and by the fall the advanced stage of infection causes deep lesions in the fleshy root of the carrot. A thick mycelial weft covers the root and presents a characteristic purplish

discoloration. Lesions develop on the carrot which at first are small but soon enlarge and coalesce and give to the infected part of the carrot the appearance of gross decay (Fig. 1). In the infected region there develop many minute infection bodies, which Prillieux (15) termed "corps miliaires", from whose apices arise strands of hyphae which add to the mycelial weft mentioned above. Secondary roots also become diseased, and at the base of some of the larger ones infection bodies and small lesions become evident. Diseased carrots, when freshly pulled, usually have a considerable mass of soil adhering to them.

Late in the fall, the fungus grows over the shoulders of diseased carrots and continues to form a dense mat of reddish purple mycelium a short distance up the leaf petioles and for some distance over the surface of the adjacent soil. This mat of mycelium may join adjacent carrots and cause them to cling together when pulled (Figs. 3, 4, 5). Upon separation of this mass there is usually left clinging firmly to the shoulder of each carrot a dangling mat of mycelium, dark reddish purple in the center and light pink at the margins, resembling somewhat a resupinate type of basidiomycetous sporophore (Fig. 2).

Microscopic examination of this mat revealed three distinct types of hyphae. The reddish layer at the surface was composed of long hyphal strands of uniform width, with right-angled branching, and a dense pigment in the vacuoles. Beneath this layer and extending outwards to the margin of the mat there was a layer of branching hyphae of uniform width but without the reddish pigment in the vacuoles. The bottom half of the mat was tough in consistency and resembled a typical subiculum. It was composed of non-uniform, thin-walled, colorless hyphae which were many septate and resembled hyphae from a sclerotium.

In most cases the leaves of field-grown carrots do not show any symptoms of the disease. Plants grown and infected in the greenhouse sometimes wilt and may even die if infection occurs and becomes well established before the plants have matured. Mature plants transplanted from outside plots into pots in the greenhouse for experiments in which the roots were artificially inoculated did not show disease symptoms on the foliage even though the fleshy root became badly diseased (Fig. 6).

Cultural Investigations of *Rhizoctonia crocorum* DC.

Isolation from Diseased Carrots

The method found most suitable for isolating *R. crocorum* from a diseased carrot was that of Van der Lek (17). From a newly infected carrot, small pieces containing infection bodies were washed in tap water, surface sterilized for one minute in a solution of mercuric chloride and water (1/1000), and rinsed in sterile distilled water. Under aseptic conditions these pieces were mounted in pith and thin sections were cut with a razor blade. Sections cut through an infection body were transferred to slants of 1% malt agar and incubated at 25° C. for three weeks. At the end of this time typical hyphae were observed growing from some of the sections. Soft translucent infection

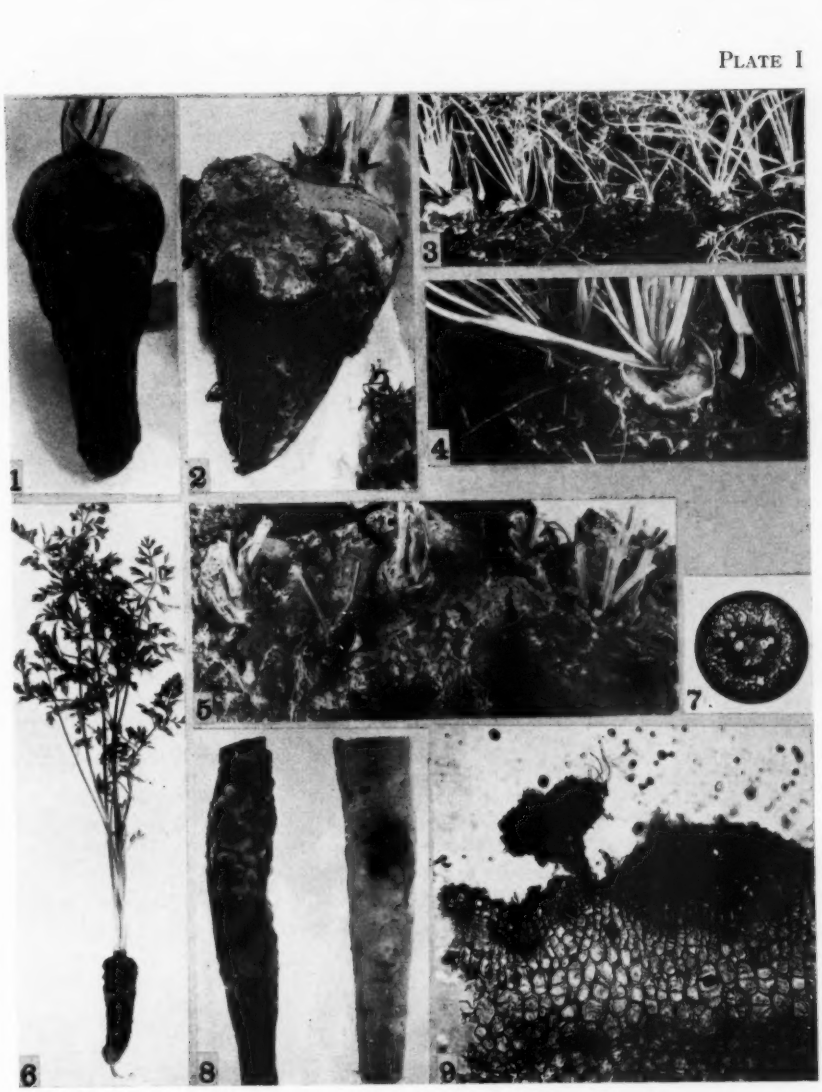


FIG. 1. A severely diseased carrot from the Thedford Marsh, Ontario.

FIG. 2. A diseased carrot showing a mat of mycelium clinging to the shoulders.

FIGS. 3 and 4. Diseased carrots shown before they were pulled. Patches of *R. crocorum* are visible on the crowns and shoulders of the plants.

FIG. 5. Mat of mycelium forming a collar around the base of the leaf petioles of diseased carrots.

FIG. 6. A Danvers Half Long carrot showing a badly infected storage root and healthy foliage.

FIG. 7. A colony of *R. crocorum* on meat-malt agar, showing large sclerotia.

FIG. 8. Carrot plugs supporting growth of *R. crocorum*. Left—cooked carrot; right—raw carrot.

FIG. 9. Photomicrograph showing the formation of a cork layer beneath two infection bodies. ×100. (The infection body on the left was partly torn away from the diseased tissue.)

bodies, designated as immature, did not grow when placed on agar, whereas mature infection bodies, that is, those which were hard and black in color, slowly produced hyphae which spread over the agar forming a colony of the fungus.

For undiscovered reasons attempted isolations using single strands of hyphae or hyphal tips failed. The aerial hyphae appeared to be depleted of contents but even hyphal tips from mycelium on newly infected carrots, as well as from old infections, failed to grow on agar, even though they had not been subjected to any adverse treatments such as surface sterilization.

Growth and Morphology on Artificial Media

Thirty-five cubic centimeters of meat–malt extract agar, as recommended by Garrett (7), were poured into Petri dishes resulting in a medium approximately 6 mm. deep. Inoculum transplants, 2 mm. square, were transferred to this medium and incubated for three weeks at 25° C. Growth on this medium consisted of a thick mat of mycelium forming a colony which was either smooth with long aerial hyphae or bunched up at the center where large sclerotia accumulated and from whose surface grew very short hyphae. In cross section the colony grew progressively thicker from the margin to the center. At the margin the colony was thin and consisted mainly of hyphae branching out over the agar. In the submarginal region a thick, tough layer began to form at the surface of the agar. This layer was composed partly of submerged hyphae and partly of compact, short-celled hyphae resembling a subiculum. In the central region of the colony the subiculum was very thick and tough.

Microscopic examination of colonies 12 weeks old revealed three types of hyphae. The first consisted of tufts of dark red aerial hyphae, unhealthy and somewhat distorted, with dark red cells which seemed to be full of a resinous substance. The tips were frequently collapsed and transparent (Fig. 15). In the second type, the hyphae formed a smooth mat of mycelium below the long reddish tufts mentioned above. This mat was buff colored and could easily be scraped away from the subiculum. En masse the hyphae of this mat displayed a waxy texture while individually they appeared healthy, were uniform in width, regularly branched, and, for the most part, not wrinkled (Fig. 16). The third type consisted of hyphae from the subiculum or bottom layer of the colony. The walls of these hyphae were thin and the cells about the same thickness as those of the second type. En masse they formed a mat of densely aggregated hyphae which branched frequently and had short cells as compared with the aerial hyphae. Individually, the strands were nearly transparent, although some were slightly buff colored (Fig. 17).

Growth Response in Relation to Type of Substrate and Host

On malt agar a variable type of growth appeared during the early experiments on cultural differences. Inoculum transferred from the original isolate grew into colonies which varied in shape and color so distinctly that six different types were detected. Inoculum from each type was transferred to

malt agar and incubated at 25° C. for three weeks. The differences were persistent from the standpoint of colonial morphology. Further subcultures of these six types revealed only two which remained stable when grown on malt agar and meat–malt agar. Of these two, one produced a fluffy mass of aerial hyphae, while the surface of the other colony was smooth and the hyphae were closely appressed to the surface. Tests on the pathogenicity of these two types showed that the latter was less pathogenic than the former one. Because of its stability and pathogenic capability the fluffy-colony type was used in all the experiments to follow.

Growth on Differential Media

Inoculum transplants of *R. crocorum* were transferred to the following media in Petri dishes: meat–malt agar (lemco beef extract 20 gm., malt extract 60 gm., agar 20 gm.); plain agar (agar 2.5%); oatmeal agar (oatmeal 100 gm., agar 17 gm.); carrot–dextrose agar (peeled and sliced carrots 200 gm., agar 18 gm., destrose 10 gm.); yeast extract agar (yeast extract 25 gm., agar 20 gm., potassium dibasic phosphate 40 gm., sodium chloride 0.2 gm., magnesium sulphate 0.2 gm., calcium carbonate 3 gm., dextrose 10 gm.); 1% malt agar (malt extract 10 gm., agar 17 gm.). The respective compounds for each of the above media were added to one liter of distilled water.

The diameters of the colonies from five replicates on each type of medium were measured at weekly intervals for three weeks after initial inoculum pieces, approximately 2 mm. square, had been transferred to the plates from a colony three weeks old. The results are summarized in Table I.

TABLE I

DIAMETER IN MILLIMETERS OF COLONIES GROWN FOR THREE WEEKS ON SEVEN DIFFERENT MEDIA

Medium	Age, weeks		
	1	2	3
Meat–malt agar	23.4	48.8	70.3
Plain agar	25.8	45.8	68.0
Oatmeal agar	—	42.0	67.0
Carrot–dextrose agar	—	38.3	58.0
Malt agar (1%)	17.2	35.8	51.3
Yeast extract agar	18.3	36.5	44.5

Meat–malt agar provided the best medium for growth. Typical colonial formation, as exemplified by a thick mat of reddish brown mycelium and sclerotia growing on a tough subiculum, resulted on meat–malt agar, carrot–dextrose agar, 1% malt agar, and yeast extract agar. The colonies on meat–malt agar contained many more sclerotia than did those on the other three

types. A variation in the colonial growth habit occurred on plain agar and on oatmeal agar. On plain agar hyphal growth was sparse, submerged, and did not build up around the inoculum piece, but spread out in long, colorless strands. On oatmeal agar a flat center, 17 mm. in diameter, was surrounded by pink, fluffy hyphae in two concentric rings 11 mm. wide which, in turn, were surrounded by a smooth, grayish, submarginal mycelial mat bordered by two concentric rings of chocolate brown hyphae. On malt agar, meat–malt agar, yeast extract agar, and carrot–dextrose agar, *R. crocorum* imparted a dark brown pigment which diffused into the agar but which was not evident on oatmeal agar and plain agar.

This experiment revealed differences in colonial formation which varied according to the medium used. Probably because of its high nitrogen content the meat–malt agar supported the best growth. The appearance of so many sclerotia on this agar and of very few on malt agar and carrot–dextrose agar and none at all on plain agar does not agree with Garrett's (8) postulation that sclerotial formation is a function of a high C/N ratio.

Growth on Carrot Plugs

Five mature carrots, about three quarters of an inch in diameter, were surface sterilized with mercuric chloride (1/1000), washed with sterilized distilled water, and inserted into sterile test tubes 1 in. in diameter. Five more carrots were placed in test tubes and sterilized in a pressure cooker. The 10 carrots were then inoculated on the unbroken surface 2 in. from the upper end and were incubated at 25° C. Each carrot rested on a piece of moistened filter paper placed in the bottom of the tube as a precaution against drying out during the experiment.

After three weeks, on the cooked carrot plugs the fungus had spread an inch on either side of the point of inoculation and the type of colony formation was similar to that on meat–malt agar. So many large sclerotial masses formed that finally the whole colony became bumpy. Growth on the raw carrots was much slower and less profuse: only a few hyphal strands spread sparsely over the carrot. After 30 days, in both cases, growth had further increased (Fig. 8). On the raw carrot, fluffy masses of hyphae appeared close to the inoculum piece; the long radiating strands had increased in length but no infection bodies were noticeable until after 37 days. There were no infection bodies at any time on the cooked carrots.

The difference in growth in the two cases was likely due to the relative availability of nutrients. In the case of the cooked carrots this availability provided a very rapid growth of *R. crocorum* in its saprophytic phase. As suggested later, saprophytism is well established in this fungus, while its parasitic capability is dependent upon a biotic potential characterized by a high energy level which appears to be required for the initiation of infection.

Innate Properties of Growth According to Quantity of Inoculum

Four each of 10 differently sized inoculum pieces having diameters of 1, 2, 3, 5, 7, 8.5, 10, 11, 13, and 15 mm. were cut and transferred from young actively

growing cultures to meat–malt agar in Petri dishes, and incubated at 25° C. for 52 days. At approximately biweekly intervals measurements were recorded of the diameter of each colony.

After 10 days of incubation the width of the colonies increased with an increase in size of inoculum piece. After 24, 38, and 52 days of growth there was still an increase in width of the colonies with increase in inoculum size but it was not as nearly linear as it was after 10 days. The colonies produced by pieces of inoculum 1 and 2 mm. in diameter after 52 days equalled or exceeded in diameter the colonies produced by inoculum pieces 7, 8.5, 10, 13, and 15 mm. in diameter, whereas before this time the former colonies were always narrower than the rest. The widest colonies grew from inoculum pieces 11 mm. in diameter.

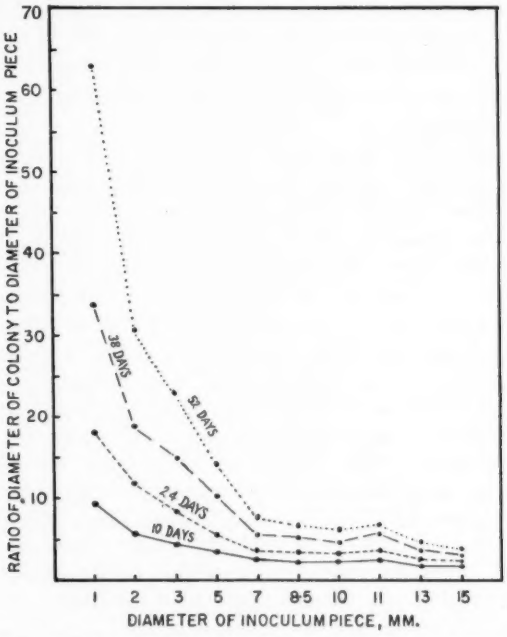


FIG. 10. The effect of size of inoculum piece on the growth efficiency of *R. crocorum*.

Fig. 10 shows that the efficiency of growth of this fungus, as determined by the ratio of the size of the colony produced to the size of the inoculum piece, was greatest when small rather than large pieces of inoculum were used. After 52 days of growth on agar a 1 mm. inoculum piece produced a colony 63 times its width, whereas a 15 mm. inoculum piece produced a colony only 3.4 times its width. Since the smaller pieces of inoculum produced the most uniform colonies, these pieces were used for producing inoculum for all the experiments on pathogenicity tests.

Growth on Meat–Malt Agar at Different Temperatures

Pieces of inoculum 2 mm. in diameter from a culture three weeks old were transferred to 70 plates of meat–malt agar. These were divided into groups of 10, each of which was incubated for three weeks at a different temperature. To determine the effect of temperature on the growth of the fungus, measurements were made of the width of each colony.

A maximum growth of 44.6 mm. occurred at 25° C. and growth decreased rapidly at temperatures above 30° C. until at 35° C. the diameter of the colonies was only 4 mm., while at temperatures below 25° C. growth decreased proportionately as the temperature decreased until, at 5° C., the width of the colonies was approximately 4 mm. At 5° and 35° C. the fungus did not begin to grow until the third week of incubation.

Factors Affecting the Parasitic Activity of *Rhizoctonia crocorum*

Materials and Methods

Carrots of the variety Danvers Half Long were used in all the following experiments on parasitism. For inoculation purposes, the fungus was grown at 25° C. for three weeks on meat–malt agar. Seedling carrots were grown in the greenhouse for pathogenicity tests on seedlings and, for experiments in which older carrots were needed, outside plots were maintained on light sandy-loam soil at Harrow, Ontario. When steam-sterilized muck was employed instead of naturally infested muck, inoculum either was mixed with the muck or was pressed against the surface of each carrot. All the experiments were allowed to run for six to eight weeks from the time of inoculation.

In the outside plots a record of the soil temperature at depths of 1 and 5 in. was kept by means of a Taylor Centennial Soil Temperature Recorder.

Depending upon its severity, disease incidence was estimated by two methods. The "squared paper" method was used when the disease from direct surface inoculation was not extensive, whereas the "infection rating" method was used where the inoculum was incorporated into the muck, and in cases of severe disease incidence following surface inoculation of the carrots. When the former method was used, the estimate of the amount of disease in each test was based on the average area over which the fungus had spread on 10 carrots at the time of examination. The area involved was outlined with ink and measured by tracing the outline on a piece of squared paper of which the area of each square was known. The "infection rating" method was based on a comparison of diseased specimens with diagrams on a disease rating chart (Fig. 11) constructed with reference to 10 diseased carrots chosen to represent 10 different degrees of infection from less than 4% to 100%. A disease rating of 1 thus represented a carrot whose percentage of diseased surface was within the limits of 0.1–4.0% (Table II).

The Influence of Type of Inoculum on Its Parasitic Capability

A study of the morphology of *R. crocorum* revealed different types of hyphae both from colonies grown on artificial media and from the fungus growth on

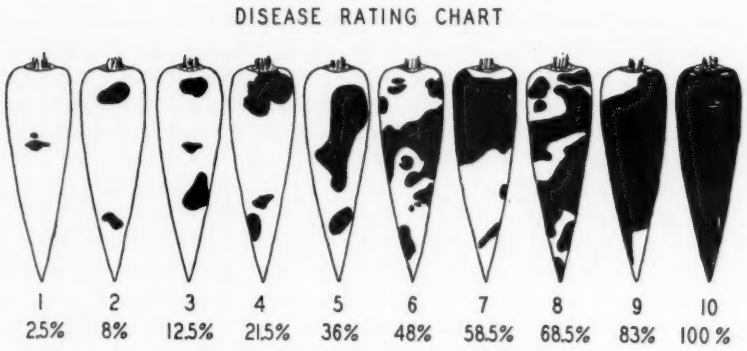


FIG. 11. Disease rating chart showing diagrammatically the reference used for estimating the percentage of surface infected and the corresponding disease ratings of the diseased carrots.

TABLE II

TABLE OF FIGURES FOR DISEASE RATING AND THEIR COMPARABLE FIGURES FOR THE RANGE OF PERCENTAGE OF DISEASE FOR EACH RATING

Disease rating	Percentage of disease	Disease rating	Percentage of disease
0	Less than 0.1	6	42.1 – 53.0
1	0.1 – 4.0	7	53.1 – 63.0
2	4.1 – 10.0	8	63.1 – 76.0
3	10.1 – 17.0	9	76.1 – 90.0
4	17.1 – 28.0	10	90.1 – 100.0
5	28.1 – 42.0		

living carrots. On agar three main types were encountered: fluffy, buff to reddish colored aerial hyphae which formed a mat of mycelium over the surface of the colony (Fig. 15); short-celled, irregularly-branched, thinwalled, and nearly colorless hyphae which formed a tough subiculum next to the agar (Fig. 16); and individual sclerotia, formed on the top of the colony and usually near the center (Fig. 7). To discover the part of the colony most effective in parasitizing living carrots, an experiment was set up using carrots 14 weeks old and, as a source of inoculum, colonies of *R. crocorum* four weeks old. Under aseptic conditions, masses, measuring approximately 2 mm. square, of each of the three types of hyphae were removed from a colony and immersed in droplets of water in sterile Petri dishes. Three hours before inoculation one row of carrots in the field plot was watered to ensure a moist environment for the inoculum once it was applied to the carrots. Each type of inoculum was then transferred to 10 carrots all of approximately the same size. The carrots were inoculated 2 in. below the crown and watered again, after which water was applied every other day for the first week so that the

small masses of inoculum would not dry out. This was followed by a regular schedule of watering twice each week. The carrots were inoculated on August 26, 1952, and, by use of the squared paper method, were assayed for disease on October 21.

Infection occurred from the subiculum and sclerotia but not from the aerial hyphae. Greatest infection resulted from masses of hyphae from the subiculum.

These results parallel closely those obtained with respect to the growth when these three types of inoculum were transferred to a meat–malt agar medium. The aerial hyphae did not grow at all, the sclerotia grew very slowly, in most cases merely increasing in size rather than spreading over the agar, while even small bits of hyphae taken from the subiculum grew rapidly and formed normal colonies within a few weeks.

The Influence of Quantity of Inoculum on Its Parasitic Capability

Since small pieces of inoculum were found to be more efficient in producing colonies on agar than larger pieces, the question arose regarding a possible relationship between the parasitic capability of this fungus and the size of the inoculum piece. On August 19, 1952, 100 carrots, 13 weeks old, were inoculated in groups of 10 in the field plot, approximately two inches below the crown, with inoculum pieces of the following sizes: 1, 2, 3, 5, 7, 8.5, 10, 11, 13, and 15 mm. in diameter. The watering schedule was the same as that mentioned in the preceding experiment, and the carrots were harvested and examined for disease on October 15.

As regards infection capabilities the data indicated an increase nearly proportionate in effectiveness with the size of the inoculum piece until a diameter of 11 mm. was reached. There was no further increase beyond that diameter. The diseased areas in square centimeters corresponding to the above inoculum sizes were as follows: 0.2, 0.4, 3.0, 8.8, 8.6, 18.7, 22.8, 11.1, and 22.1. Inoculum pieces 1 mm. and 2 mm. in diameter produced only a few typical long strands of hyphae close to the inoculum piece, but did not show evidence of infection bodies or lesions. However, infection bodies and lesions occurred regularly on the carrots inoculated with inoculum pieces greater than 3 mm. in diameter. By far the most severe infection resulted from inoculum pieces from 10 to 15 mm. in diameter. This observation suggests that the larger inoculum pieces were better equipped with stored food for maintaining aggressiveness while primary infection was becoming established.

These results do not agree with those related to growth on an artificial medium where, after 52 days, the small pieces of inoculum produced colonies equally as large as or larger than those from some of the larger-sized inoculum pieces. The greater difficulty encountered in the establishment of this fungus on a living host than on artificial media suggests that low concentrations of readily utilizable nutrients must characterize the host in the living organized state. In the absence of available food, *R. crocorum* cannot rapidly initiate infection and, until infection becomes well established, the supply of food appears to be a limiting factor of further growth. In large pieces of inoculum

on the contrary, there is enough reserve energy to promote the luxuriant growth of mycelium which is necessary to initiate primary infection.

Influence of the Place of Inoculation on the Parasitic Capability of R. crocorum

In his review of sugar-beet diseases, Hull (10) stated that violet root rot may occur on any part of the root, but most frequently attacks the tail first and spreads from there evenly up the tap root. Hull and Wilson (11) showed that sclerotia placed in both deep and shallow layers of the soil may cause infection. In the present investigation carrots have been found on which infection appeared to have been initiated near the shoulder, the tail, and at intermediate points. Thus, *R. crocorum* seems to be active at various depths in the soil and able to attack any part of the fleshy carrot root.

To determine the most susceptible point on a mature carrot and to find out if the fungus spreads upward from the tail more quickly than downward from the shoulder, on August 29, 1952, 90 carrots 14 weeks old were inoculated in a field with inoculum three weeks old. Thirty carrots were inoculated at each of the following loci: on the shoulder 1 in. below the crown, midway between the crown and tail, and 1 in. above the tail, these points being designated top, middle, and bottom, respectively. At each point three methods of inoculation were used: inoculum pressed against the smooth, unwounded surface; inoculum placed over the point of secondary root emergence; and inoculum pressed against a wounded surface made by slicing away a piece of the periderm. These methods were designated "smooth surface", "natural wound", and "artificial wound" inoculations, respectively. Ten carrots were used for each method of inoculation, totalling 30 for each place of inoculation. The water schedule was the same as in the previous experiments. Sixty-six days later the carrots were dug and rated for disease by the squared paper method.

Infection occurred at all three places of inoculation but the diseased area tended to be greatest at the center of the carrot. Application of the inoculum piece to the smooth surface resulted in a larger number of infections than inoculation of a natural or an artificial wound. Inoculation on a natural wound 1 in. from the tail caused only three carrots out of 10 to become diseased, while no infection at all occurred on those inoculated on an artificial wound in the same region. The results support previous observations that *R. crocorum* can infect any part of a carrot, and they show that an unwounded surface provides a fertile infection court.

While maximum growth was directed towards the region midway between the top and center, analyses of the data secured from the test showed that none of the apparent differences were significant at the 5% level of significance. Therefore, it was concluded that the fungus can spread in all directions over the carrot no matter where the primary infection occurs.

The Effect of Age of the Carrot on Parasitism of R. crocorum

Baudyš (1) reported that *R. crocorum* attacked the carrot from the earliest stage of its development and could kill the carrot within two or three weeks.

He referred to this particular strain as that of Eriksonn's specialized form *dauci*. In the present investigation, the disease has never been observed on seedling carrots grown in naturally infested muck. But while the fungus in the Thedford Marsh in Ontario seems not to attack seedlings, it does attack older carrots regardless of their size. To investigate this factor of age, controlled experiments were set up in the greenhouse and in outside plots to compare the susceptibility of seedlings and mature carrots.

In a preliminary greenhouse experiment in which carrots of different ages were inoculated on the roots and hypocotyls and later examined for disease, evidence was obtained that carrots must reach a certain state of maturity before the fungus can attack and become established parasitically. Seedlings up to six weeks old were almost nonsusceptible, while carrots over six weeks old were very susceptible.

To expand the above observations, an experiment was designed to test the effect of the age of carrots on their susceptibility under field conditions. Beginning on June 19, 1952, when the carrots were four weeks old, and continuing at 14-day intervals until September 11, sufficient carrots in the outdoor plots were inoculated to provide 10 carrots for examination every two weeks subsequently until harvest time. Inoculum transfers 7 mm. in diameter from cultures three weeks old were applied 1 in. below the crown in all inoculations. The plants were watered before inoculation and twice each week through July, August, and September. At the time of examination, the soil was carefully removed from around the top half of the carrot so as not to disturb the inoculum, and the carrot was pulled by hand. In nearly every case, whether or not infection had occurred, the inoculum remained in contact with the carrot. The amount of disease on the carrots in the different series was determined by the squared paper method, and the area diseased was recorded in square millimeters. A record of the soil temperature at depths of 1 and 5 in. was kept by means of a Taylor Centennial Soil Temperature Recorder. The results of this experiment are shown in Figs. 12 and 13.

Infection did not occur on the carrots in series 1 and 2 which were inoculated June 19 and July 3, but in series 3 infection was apparent on July 31 and by August 14 it was clearly established (Fig. 12). In series 4 to 7 the carrots all became diseased and the diseased area increased during the first four weeks following inoculation. Thus infection did not occur until late July or early August by which time the carrots were at least eight weeks old, and subsequently infection occurred in every series following the inoculations.

From July 7 to September 14 the average soil temperature at both the 1- and 5-in. levels in the field plot was above 20° C. (Fig. 13). From July 17 to July 31, during which time infection was initiated in series 3, the average soil temperature was 27° and 26.1° C. at depths of 1 and 5 in., respectively. In the remaining series, infection occurred and spread rapidly while the temperature decreased considerably. Since no infection occurred in series 1 and 2, it was thought that the soil temperature might have been too high when those carrots were inoculated to permit disease development. To check this

possibility two further groups of seedlings were inoculated on September 3 and 16 when they were four and six weeks old, respectively. Although these were examined on three subsequent occasions, no infection was found.

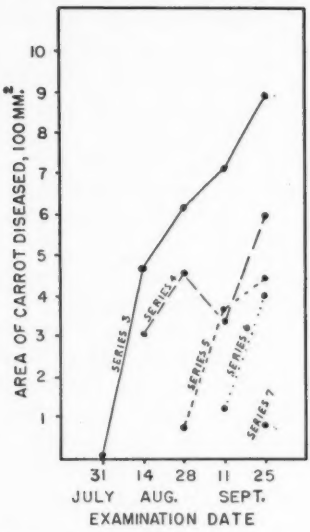


FIG. 12. Amount of disease on the samples of carrots in each series at the time of each examination.

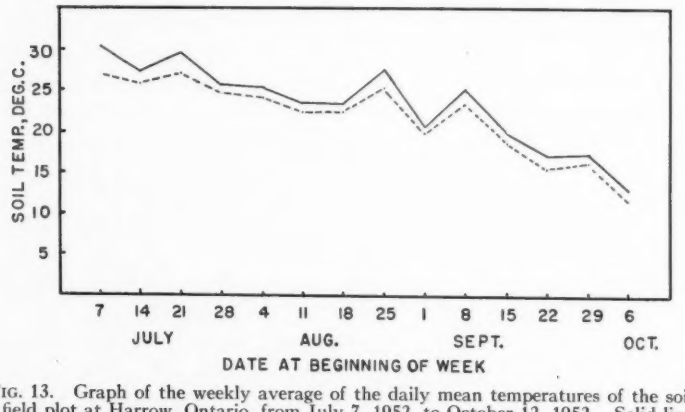


FIG. 13. Graph of the weekly average of the daily mean temperatures of the soil in the field plot at Harrow, Ontario, from July 7, 1952, to October 12, 1952. Solid line at the 1 in. level; broken line at the 5 in. level.

This experiment was carried out under the same conditions of temperature and moisture as obtained for series 5, 6, and 7 of the last experiment within which nearly all the older carrots became infected and the infection continued

to spread. It is apparent therefore that temperature could not have inhibited infection on the seedlings and that their age was apparently the limiting factor.

The following summer a similar experiment was carried out in naturally infested muck in the Thedford Marsh. Carrot seed was sown on May 28 and the carrots were examined for disease every two weeks until harvest, October 15. The temperature of the muck was recorded at depths of 2 in. and 10 in.

There was no evidence of disease on July 20 when the carrots were seven and one half weeks old. On August 5, when the carrots were 10 weeks old, traces of violet root rot were observed on 20% of the carrots examined, and from this time until harvest the disease increased both in severity and the percentage of carrots diseased. As the temperature from June to August proved not to limit disease development, these observations support the evidence from the previous experiments that carrots must reach a certain age before they become susceptible to violet root rot.

In her description of the morphology and anatomy of a carrot, Esau (5) observed that very young carrots have a cortical layer surrounding the stele and, as the roots get older, the increase in thickness results in loss of the cortex which, in older storage roots, is entirely replaced by a periderm. She determined the age at which an actively growing seedling bursts the cortical layer and begins forming a cork layer around the outside to be approximately 39 days after the seed had been sown and approximately 35 days after emergence of the cotyledons above ground. This is approximately the time when the plants become noticeably susceptible to violet root rot. This susceptibility may result from the formation of periderm which provides an easy passage for the fungus into the carrot. As shown later, penetration occurs by a hyphal tip forcing its way between the cells of the periderm. The cells of the intact epidermis of a seedling carrot are smooth and uniform at the points where they join, whereas the surface cells of the periderm of an older carrot are not uniform and tend to slough. These latter conditions furnish openings that are easily accessible to entrance by the tip of a hyphal strand (Fig. 23).

The Influence of Temperature on the Development of Infection Bodies and Lesions

Regardless of the date of primary infection, infection bodies and lesions did not appear on diseased carrots in any of the inoculation series in the field plot at Harrow before September 25 at which time the carrots were 18 weeks old. In series 3 to 6, at least two weeks elapsed after inoculation before these formations occurred. In series 7, infection bodies and lesions were only just beginning to show two weeks after primary infection. However, in the Thedford Marsh the following summer, infection bodies and deep lesions on diseased carrots were first observed on August 17 when the carrots were approximately 12 weeks old. This was approximately six weeks earlier than they appeared on the carrots in the experimental plot at Harrow. Both occurrences corresponded with a range of soil temperatures nearly optimum for the best development of the disease. The temperature in the Thedford muck from August 3 to August 23, 1953, was approximately the same (average

19.0° C.) as that of the Harrow soil from September 15 to September 29, 1952 (average 17.8° C., Fig. 12), whereas the temperature of the Harrow soil from August 4 to August 25, 1952, was well above the optimum for disease development (average 24.0° C.). These data suggest that, once infection has occurred, there is a relationship between the severity of the disease and temperature.

The Effect of Soil pH on Disease Incidence and on Carrot Growth

While Hull and Wilson (11) have presented evidence indicating that severe infections of violet root rot occur mainly on alkaline soils, in the Thedford Marsh in Ontario the muck soil in which carrots have shown severe infection is very acid. In 1949 the pH of such a field of muck was 6.5, while the acidity had increased to pH 6.0 in 1950 and to pH 5.8 in 1951, and each year the disease was severe in scattered patches in this field. In 1952 another field, which was growing its second crop after having been newly broken in 1951, produced severely infected carrots in patches of muck whose pH was 5.7. Such observations are obviously not in agreement with those of Hull and Wilson. To discover more about the effect of pH on this disease an experiment was set up in the greenhouse using two types of muck: (1) Peters muck, a black, coarse, crumbly one from the Cranberry Marsh south of Harrow, Ontario, whose pH was 7.1; (2) Klondyke muck, a dark brown, fine, and powdery one from the Thedford Marsh, with a pH of 5.7.

On September 9, 1952, 27 10-in. pots filled with steam-sterilized Peters muck were arranged into nine groups of three pots each, four of which were treated with flowers of sulphur at four different rates to increase the acidity and four other groups with four different rates of sodium carbonate to induce an alkaline reaction. The remaining group of three pots was given no treatment. The muck was kept moderately moist in the greenhouse where the mean temperature was 68° F., and was left standing for 23 days to allow an equilibrium to become established.

On October 2, two pots of muck from each group were artificially infested with *R. crocorum* grown for three weeks at 25° C. Six colonies, approximately the same size, were chosen for each pot, cut into small pieces, and thoroughly mixed with the muck. Five mature carrots from the outside field plot were transplanted into each of the 18 pots of muck containing the inoculum, and were allowed to grow until November 17 when they were all harvested and examined for disease, comparisons being made by the infection rating method. The third pot in each group was not infested with *R. crocorum* but was planted with carrot seed to test the effect of pH on the growth of carrots. Enough water was added throughout the duration of the test to keep the muck moderately moist. By immersion of Beckman pH Meter electrodes into moist muck, the pH of the muck in all the pots was determined when the carrots were planted and again when they were harvested.

A similar procedure was used with the naturally infested Klondyke muck, except that no inoculum was applied, no test was run on the effect of pH on

the growth of carrots from seeds, and only seven days were allowed for the muck to reach a state of equilibrium after the chemicals were applied.

The results of this experiment, which are presented in Table III, show that *R. crocorum* can infect carrots over a wide pH range. In the artificially infested muck the disease was most severe in the acid range, and the heaviest infection occurred within the pH range of 6.3 to 5.5 which was attained by the addition of sulphur at the rate of 1000 lb. per acre. Although infection in the naturally infested muck was low as compared with that in the artificially infested muck, it did occur over the entire range of pH values from 3.6 to 7.8 with the greatest infection occurring within the pH range of 6.1 to 5.7.

The carrots grown from seed in Peters muck grew over the entire pH range, although growth was better in the acid than in the alkaline range and was best within the pH range of 6.5 to 5.5.

These results indicate that the optimum pH values for carrot growth, disease development, and vegetative development of *R. crocorum* in artificial culture are closely coincident and within a very narrow range of pH values of approximately 5.5 to 6.5.

The Effect of Soil Temperature on Disease Incidence, Growth in Culture of R. crocorum, and Carrot Growth

Preliminary experiments conducted in constant temperature chambers at temperatures of 6°, 12°, and 20° C. indicated that *R. crocorum* could infect carrots over a wide range of temperatures.

To check the cardinal temperatures for the growth of carrots, for infection, and for the growth of *R. crocorum* in culture, Wisconsin constant temperature tanks were employed, one being held at each of the following temperatures: 30°, 25°, 20°, 15°, 10°, and 5° C. On November 4, 1952, the eight cans in each of these tanks were treated as follows: one was used as check on the development of seedlings grown from seed in sterilized muck; two cans of seedlings and two of mature carrots were artificially inoculated; two cans of seedlings and two of mature carrots were grown in naturally infested soil; one can containing meat–malt agar cultures of the pathogen was subjected to the same temperature. Sufficient water was added throughout the duration of the experiment to maintain the muck in a moderately moist condition. Vermiculite was added to a depth of ½ in. as insulation between the temperature of the muck and the air temperature of the greenhouse.

The carrot seed was planted at the rate of 50 seeds per can and one month after germination the plants were thinned, leaving approximately 10 plants in each can. Observations were made on the date of emergence at each temperature; the height of the foliage after one month; the height of the foliage, length and width of the fleshy roots, and average weight per carrot at the time of harvest, February 24, 1953. Seedling carrots were planted at the rate of six per can. On December 10 three seedlings were pulled from each can and examined for infection. The remaining plants were examined on January 6, 1953. The mature carrots were brought in from the field plot and planted at the rate of five per can. To those that were artificially

TABLE III

DISEASE DEVELOPMENT AT NINE DIFFERENT pH LEVELS IN TWO TYPES OF MUCK

Treatment, lb. per acre	Peters muck (artificially infested)					Klondyke muck (naturally infested)				
	pH		No. carrots infected out of 10	Disease rating	Total surface diseased, %	pH		No. carrots infected out of 10	Disease rating	Total surface diseased, %
	Initial	Final				Initial	Final			
Sulphur										
4000	5.1	3.5	10	8	70.0	4.8	3.6	3	1	2.0
3000	5.6	4.6	10	8	71.0	5.1	4.0	5	2	5.5
2000	6.1	5.0	10	5	37.4	5.4	4.8	6	2	5.5
1000	6.3	5.5	10	10	99.7	5.4	5.1	4	1	3.0
No treatment	7.1	6.6	10	7	58.5	5.7	6.1	8	4	21.5
Sodium carbonate										
1000	7.7	6.8	10	9	82.9	6.5	6.4	7	2	8.6
2000	8.0	7.4	9	6	43.9	7.1	7.2	8	3	10.7
3000	8.5	8.0	10	5	36.1	7.6	7.3	8	3	17.7
4000	8.6	7.9	10	5	41.0	7.8	7.8	3	2	8.4

inoculated 11 mm. fungus transplants from a four-weeks-old culture were applied to the smooth surface. All the mature carrots were harvested on January 5, 1953, and examined for disease by the infection rating method.

The seed planted in the noninfested muck germinated and produced healthy seedlings at all six temperatures, although the time taken for germination and the appearance of the cotyledons increased with decreasing temperatures. At the end of 21 days, a soil temperature of 30° C. proved to be the best for foliage development. After 16 weeks' growth, the tops of the carrots were all approximately the same height, except at 5° C. where they averaged 3 in. shorter than the rest, but the leaves at 5° and 10° C. were much stronger and more erect than those at the higher temperatures. The carrots most uniform in size and having the best shape and color as well as the best yield, in terms of the average weight of each carrot, grew at a temperature of 25° C. At 30° C. the carrots were short and stumpy, at 10° C. they were long and narrow, while at 5° C. they were bleached, narrow, and produced a large number of secondary roots. The seedling carrots, which had been transplanted into naturally infested muck, were infected only at 20° and 25° C. and even at these temperatures only four carrots out of 24 examined were diseased.

TABLE IV

EFFECT OF TEMPERATURE ON INFECTION OF MATURE CARROTS PLANTED IN NATURALLY-INFESTED MUCK AND IN ARTIFICIALLY-INOCULATED STEAM-STERILIZED MUCK. THE FIGURES REPRESENT THE AVERAGE DISEASED AREA ON 10 CARROTS

Temperature, ° C.	Naturally-infested muck		Artificial inoculations in steam-sterilized muck	
	Disease rating	% infection	Disease rating	% infection
30	0	0	1	1.1
25	3	10.8	8	64.3
20	4	20.5	8	75.9
15	1	2.4	4	23.1
10	1	0.2	4	17.3
5	0	0	1	0.5

Table IV shows the results from an examination of the mature carrots after they were harvested. In naturally infested muck, infection was greatest at 20° C., while very little infection occurred at 15° and 10° C., and none at all at 30° and 5° C. On the artificially inoculated carrots, infection occurred at all temperatures, was greatest at 20° C., and was hardly noticeable at 30° or 5° C. Although the percentage of infection was highest in both cases at 20° C., on the artificially inoculated carrots the disease rating at 20° and 25° C. was equally high, and nearly equal in the naturally infested muck where the difference in disease ratings at 20° and 25° C. might have been due to differences in inoculum potential.

Growth of *R. crocorum* on the meat–malt agar in Petri dishes paralleled closely the figures shown previously under the heading *Growth on Meat–Malt Agar at Different Temperatures.*

In general, there was a close agreement among the temperatures which are best for the growth of the fungus, for the growth of the host, and for maximum infection (Fig. 14). The fungus was pathogenic throughout the range of temperatures at which it grew, except at 30° C. where infection was slight even though the fungus still grew well in culture. This slight infection must have resulted from loss of pathogenicity of the fungus at this temperature because the other variables were under reasonable control. Both the curve

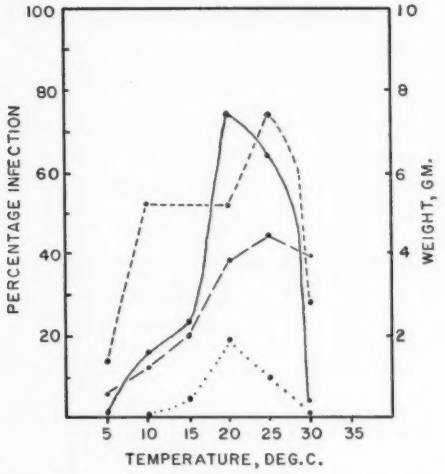


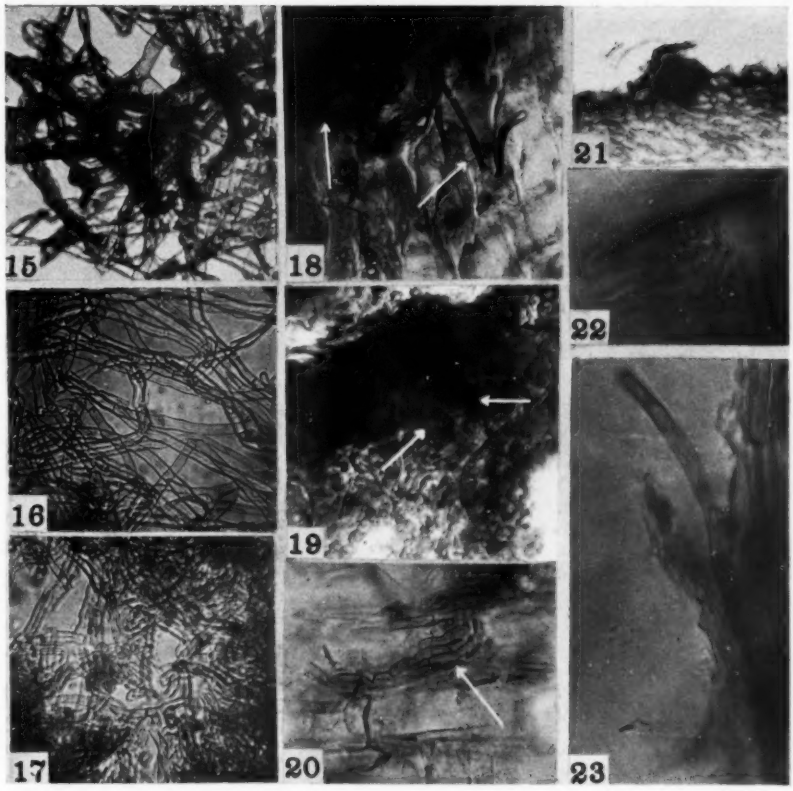
FIG. 14. Influence of temperature on disease incidence, growth of *R. crocorum* in culture, and carrot growth expressed in terms of weight. Disease (inoculated) ————; disease (natural infestation) ·····; fungus growth — — — —; weight of carrots – – – – – –.

of disease incidence and the curve of fungus growth rise with essentially the same rapidity to 20° C. but the fungus growth continues to increase between 20° and 25° C. Thus there is an interesting temperature range between 20° and 25° C. in which the previously close correlation between pathogenicity and vegetative growth breaks down and, at temperatures which are essentially optimum for both fungus and host, disease incidence falls very sharply.

Host–Parasite Relations

Colonization of the Host

Microtome sections 50 microns thick were cut from diseased tissue, mounted in lactophenol and cotton blue, and examined for the presence of *R. crocorum.* Initial entry of the fungus into the host tissue is accomplished by single hyphal strands forcing their way mechanically between the cells of the outer periderm



FIGS. 15–17. Three types of mycelium taken from a colony of *R. crocorum* grown on meat–malt agar for 12 weeks. ×300.

FIGS. 18–19. Cross sections through the periderm of a diseased carrot showing intercellular hyphae indicated by the arrows. ×300.

FIG. 20. Cross section showing intracellular hyphae. ×300.

FIG. 21. An infection body which has forced out of place a fragment of the periderm.

FIG. 22. The small mass of intracellular hyphae has caused the cell immediately above it to collapse.

FIG. 23. An external hyphal strand entering the carrot tissue by forcing apart the outer cells of the periderm.

(Fig. 23). Following penetration, both intercellular and intracellular hyphae are formed (Figs. 18, 19, 20). The intercellular hyphae are hyaline and narrow in cross section, whereas the intracellular ones are yellowish in color, short-celled, uniformly convoluted, and with sclerotia-like masses formed inside the cells. The intercellular hyphae stained blue and appeared to function mainly in spreading the fungus throughout the infected tissue. Relatively large yellow areas in the periderm marked the location of yellowish intracellular hyphae, filled with oil globules, which would not react with the blue stain. However, although not all the cells in such an area were invaded by the fungus, the protoplasts were filled with a yellowish substance containing numerous oil globules. Such cells appeared to be plesionecrotic and their breakdown seemed to be due either to action in advance by the intercellular hyphae or to the breakdown of cells that were invaded.

In the sections examined, most of the internal hyphae were observed in the periderm layer, a few in the phloem parenchyma, but none beyond the cambium towards the center of the root.

Infection Bodies

Infection bodies appeared to arise in cells of the periderm. In sections through this tissue taken at the edge of a lesion, where infection bodies were just beginning to appear, small masses of short-celled, yellowish, sclerotia-like hyphae were apparent and were beginning to crush some of the adjacent periderm cells (Fig. 22). Fig. 21 shows a section of tissue bearing small immature infection bodies above one of which is a piece of the periderm which appeared to have been forced out of position by pressure exerted as a result of the emergence of the infection body. Examination of a cross section through a lesion in which infection bodies were prevalent revealed mature infection bodies deeply submerged in a layer of necrotic tissue which was easily peeled away from the remainder of the lesion. A diagrammatic representation of such a section is shown in Fig. 24, C. The top layer of the lesion clearly surrounded the infection body which, by means of hyphal strands, was firmly attached to the layer below.

The cells of crushed immature infection bodies are nearly the same color and shape as those which form the small masses in the periderm three or four cell layers below the surface. A section through a typical infection body revealed a mass of short-celled hyphae extending from the host tissue into the emerged spherical portion of the infection body. Such a mature infection body produces numerous strands of external hyphae similar to those which cause primary infection. These hyphae become very extensive and are chiefly responsible for the thick mycelial weft which is always present over the surface of a badly diseased carrot.

The evidence presented regarding the formation of infection bodies in carrot supports that of Faris (6) who found, working with violet root rot of potatoes, that infection bodies arose as a result of infection and were not, as suggested by Prillieux (15), developed by the fungus prior to infection.

Histological Response to Infection

Following infection two types of histological sequences occurred both of which tended to check the spread of the fungus by the formation of protective layers. In the first type a cork layer developed beneath the infection bodies (Fig. 9) resulting in the formation of shallow lesions, whereas the second type

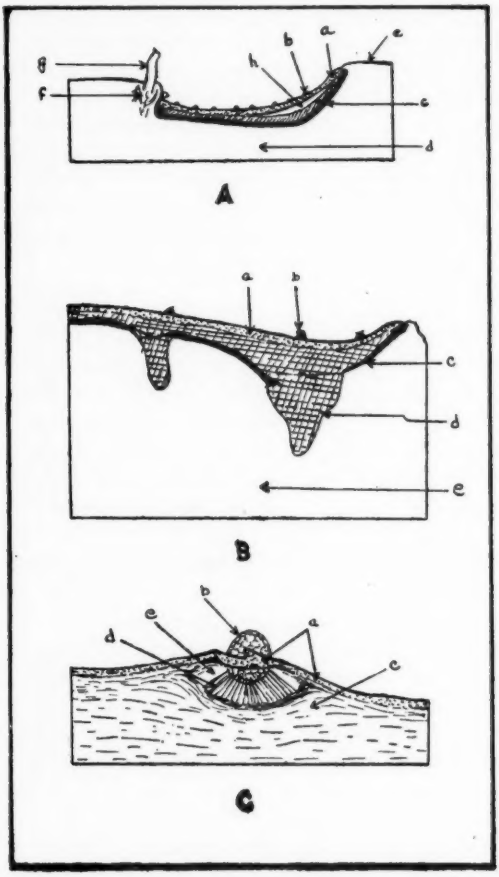


FIG. 24. Diagrammatic cross sections through lesions.

A, ×1½: *a*—top layer containing infection bodies; *b*—infection body; *c*—bottom layer of dark brown necrotic tissue; *d*—healthy tissue in region of phloem and phloem parenchyma; *e*—normal surface of carrot; *f*—point of emergence of lateral root; *g*—lateral root.

B, ×5: *a*—top layer; *b*—infection body; *c*—bottom layer; *d*—necrotic tissue which has extended deep into the phloem parenchyma; *e*—healthy tissue.

C, ×25: *a*—upper layer in which infection body is situated; *b*—infection body; *c*—necrotic tissue; *d*—hyphal strands connecting infection body to tissue of lesion below the top layer; *e*—hollow below infection body.

resulted in the formation of deep lesions. In the second case no cork barrier was formed beneath the points of infection; the fungus penetrated deeply into the phloem parenchyma, and there was a rapid breakdown of the host cells with a profuse development of internal hyphae. Thus, a lesion was formed with many large mature infection bodies on its surface, which in cross section appeared as a thick layer of dark brown necrotic tissue. Diagrammatic sections through typical lesions are shown in Fig. 24. There were two well-defined layers in the necrotic areas of such lesions. The top layer, lettered (*a*) in diagram A, was thin and porous and could be separated easily from the bottom layer (*c*) which was thick and very tough. The top layer contained the infection bodies which adhered tenaciously to the necrotic tissue when it was removed. When this layer was removed while being observed under a stereoscopic microscope, it was apparent that the infection bodies were deeply submerged in the tissue and were connected with the bottom layer by numerous strands of internal hyphae which were so aggregated as to have caused a complete breakdown of the host cells with the formation of a hollow directly beneath the infection body and between the top and bottom layers of the lesion (Fig. 24, C).

The bottom of the lesion consisted of two layers of which the upper one was tough and spongy, while the lower one was hard and somewhat darker in color than the upper one. It was this hard, dark part of the bottom layer which appeared to limit further spread of the fungus into the carrot. Internal hyphae were found in all layers of the lesion but were not evident beyond the bottom layer. However, sometimes the botton layer of a lesion failed to restrain the fungus which broke through it and continued its invasion deeper into the phloem parenchyma (Fig. 24, B).

In most cases, lesion development was confined to the carrot tissues between the lenticel-like areas from which arise the secondary roots. The cross section through such a lesion is shown diagrammatically in Fig. 24, A. The necrotic tissues in the lesion abruptly ended at the place where the secondary roots emerged, and the tissue in the region of secondary root emergence did not contain any fungus hyphae, nor did it appear to be affected by the nearness of the lesion. However, this phenomenon was not always apparent since in some cases lesions were formed in the center of which dead secondary roots appeared.

Discussion

Although *Rhizoctonia crocorum* is encountered predominantly as a root-invading parasite, there is doubt as to its parasitic capabilities in the absence of a substantial saprophytic development prior to infection. Its growth on a wide range of substrates, including even moistened filter paper, plus its lack of host specificity over a wide range, indicates an unspecialized parasitism bordering saprophytism which would seem to assure its survival.

The host range of *R. crocorum*, as far as it is known, includes mainly dicotyledonous plants with roots which develop periderm tissue at some stage in their ontogeny. In the present investigation, with carrot as a host plant,

evidence has been presented that infection does not occur unless a certain amount of inoculum is present and even then does so only after the host has reached a certain age. This stage of development is concurrent with that determined by Esau at which the carrot sheds its cortex in favor of a periderm. In hosts of this sort, therefore, the fungus can develop initially as a saprophyte on the periderm and as a result of this it acquires the capacity to launch a parasitic attack on the sublying tissues. In the initial phases of parasitic colonization, there is a curious suggestion of mass action resulting in the establishment of the parasitic phase.

The fact that in the invaded carrot tissue intracellular sclerotical bodies are formed which seem ultimately to give rise to the minute infection bodies further suggests unspecialized parasitism. Intracellular hyphae are not haustorial and lack a nutritional balance with the invaded cell which would permit each to live undisturbed in the presence of the other. Instead, as colonization occurs, the invaded cells are killed. In the organized host, the conditions are not conducive to the more rapid fungus growth that usually occurs during its saprophytic development on agar. Instead, under such conditions when nutrients are exhausted, *R. crocorum* goes into a resting stage characterized by the absence of spores and the formation of internal sclerotia.

The type and amount of inoculum required for infection is important in field practice. From colonies developed in culture only the tough subiculum and the sclerotia were able to cause disease; this fact indicates that similar structures which form in association with diseased carrots in the field late in the fall would be most important for the overwintering of the fungus and provision of the inoculum during the following summer. Apparently the long aerial hyphae of the mycelial mat which grows on the surface of the ground around the carrots and the long reddish hyphal strands which form part of the mycelial net surrounding the diseased carrot are not in any way responsible for survival of inoculum in the field, since studies of these hyphae showed them to be incapable of further growth either on agar or on living carrots in the field plot.

Furthermore, the fact that small pieces of inoculum caused less disease than larger pieces indicates that large masses of inoculum are more efficacious under field conditions. For control, therefore, cultural practices in soil management would be effectively utilized if, while working the soil after the removal of a crop, it were feasible to break up the large mats of fungus and carrot debris to ensure fine maceration of the potential inoculum. Besides the impaired parasitic capability of such small pieces of inoculum, an operation of this sort would more effectively expose the fungus to antagonistic microbiological activity in the soil.

Infection was found to occur in midsummer when the carrots had become well established in the soil. Temperature does not seem to have been the factor responsible for such late infection, since experiments revealed that throughout a wide range of temperatures the carrot develops satisfactorily and *R. crocorum* can grow in culture, initiate infection, and spread. Although

R. crocorum decreased in virulence at 30° C., the fungus and host would seldom be subjected to such a temperature in the muck of the Thedford Marsh.

Possibly the innate slowness of growth of the subiculum and sclerotia, which seem to be the only parts of the fungus which could effectively overwinter, might account for such late infection. Pieces of subiculum were found to grow slowly on agar taking as long as three weeks to form a colony 1 to 2 in. in diameter, throughout a range of temperatures from 15° to 30° C. In the soil other conditions, imposed by antagonisms, synergisms, and other associative effects, might increase or shorten the time required for effective growth of the overwintered inoculum.

The most promising explanation for this late attack under field conditions, however, seems to relate it to the age of the host. It was found that infection never occurred before the plants were about eight weeks old. This corresponds with the time when the root cortex is replaced by periderm which, it was postulated, is more adapted to the support of growth of *R. crocorum* in its initial stages of attack, and more readily penetrated by it than is the cortex.

Acknowledgments

Sincere thanks are extended to Prof. D. L. Bailey for guidance during this investigation and for his help in the preparation of the manuscript. Acknowledgement is made also to Dr. G. H. Berkeley, Officer-in-Charge, Science Service Laboratory, Plant Pathology Division, St. Catharines, Ontario, who suggested the problem, and to Dr. L. W. Koch for his interest in the problem and for the use of the facilities of the Science Service Laboratory, Harrow, Ontario.

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THE ANTIBIOTIC ACTIVITY OF SOIL MICROORGANISMS AS RELATED TO BACTERIAL PLANT PATHOGENS[1]

By Z. A. Patrick[2]

Abstract

In an attempted evaluation of the importance of soil antagonisms as a possible factor in the different survival capabilities of some bacterial plant pathogens in the soil environment, a comparison was made of the numbers of antagonists detected when different plant pathogenic species were used as test organisms in determining the "antibiotic potential" of nine "virgin" soils. It was found that there are present among the soil flora a great abundance of microorganisms intrinsically capable of antagonizing most of the bacterial pathogens tested and only for a few species are such antagonists relatively rare. There were great differences in the number of isolates antagonistic to the different pathogenic species, even in the same genus, and there seemed to be a correlation between the numbers of antagonists, as found here, and the capability of a species to maintain itself for long periods in the soil. For the most part the *Xanthomonas* species appeared to be most sensitive to the antagonistic soil microflora while the soft-rot-causing *Erwinia* species were most resistant.

A comparative study of the antibiotic activity of 120 of the most active antagonistic isolates tested against 28 bacterial plant pathogens showed that each antagonist was characterized by a specific antibacterial spectrum and those antagonists having the most intense antibiotic activity usually inhibited the greatest number of bacterial species. Many antagonists were highly specific, affecting only certain groups or even certain species. The high specificity which characterized some of the antibiotic reactions was used to separate sharply, consistently, and with minimum effort such closely related species as *E. carotovora* and *E. atroseptica* or *X. corylina* and *X. juglandis*.

Introduction

It has been observed by many investigators (18, 19, 40) that certain bacterial plant pathogens can persist in the soil while others tend to disappear relatively quickly. Why certain species even in the same genus can survive in the soil for a long time while others fail to do so has never been adequately explained. It has been shown (6), for example, that *Erwinia carotovora* and *E. atroseptica* are able to survive for long periods in a soil environment while *E. amylovora* and *E. tracheiphila* vanish very rapidly. There are many factors that must be considered in attempting to account for such differences but, since all plant pathogenic bacteria during some part of their existence are almost certain to come into contact with the soil microflora sufficiently to enable the microorganisms therein to exert antagonistic or synergistic effects upon them, there is a strong possibility that the antagonistic action of the soil microorganisms towards the plant pathogen may be of the utmost importance. To date much evidence has accumulated to support the belief that antagonisms between

[1] *Manuscript received May 20, 1954.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ontario. Part of this study was carried out with financial assistance in the form of a studentship from the National Research Council of Canada. Based on a thesis presented in November, 1952, to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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microorganisms play a major part in establishing the microbial balance in soils (1, 24, 45, 46), consequently antibiosis as a possible explanation of the behavior of some phytopathogenic bacteria in the soil becomes very intriguing and of considerable theoretical and practical importance to plant pathology. Though many phenomena of microbial behavior in soil are most readily interpreted in terms of antibiotic action (4), conclusive evidence is still lacking whether there is any relationship between the relative abundance of organisms antagonizing a given plant pathogenic species and the ability of that species to survive in the soil. Similarly very little is known of the relative sensitivity of the different phytopathogenic bacteria to the antagonistic soil organisms and the antibiotic substances produced by them. To add a little to the available information a survey was made of a few "virgin" soils to determine their "antibiotic potential" against a number of plant pathogenic bacteria, the choice being distributed so that all genera containing plant pathogens were represented. Thus species from the genera *Agrobacterium*, *Bacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas* were used as test organisms and their relative sensitivity to the antagonistic soil microflora was determined.

Materials and Methods

A. Isolation and Growth of the Bacterial Plant Pathogens Which Were Used as Test Organisms

In the sixth edition of *Bergey's Manual of Determinative Bacteriology* (3) most of the plant pathogenic bacteria are included in the genera *Pseudomonas* and *Xanthomonas*, while the remaining ones are in *Agrobacterium*, *Bacterium*, *Corynebacterium*, and *Erwinia*. A number of representative species from each of these genera were selected for the present study and used as test organisms, the relative numbers of antagonistic organisms that were present in the various soils against each specific test organism being determined. The bacterial plant pathogens used as test organisms are listed in Table I accompanied by a few details with respect to host, locality, and the person by whom they were isolated. To determine whether the different isolates of the same plant pathogenic species obtained from different localities behave differently, more than one isolate of the same species was used in most cases.

Methods of Isolation of Bacterial Plant Pathogens

The method used in isolating bacterial plant pathogens from diseased tissues was a combination of the various methods used by other investigators (9, 12, 35, 37) and will not be described in detail. Single discrete colonies which fitted the description of the pathogen in the manual by Bergey (3) or Elliot (14) were selected and streaked on fresh nutrient agar plates. If all the colonies that came up were identical they were regarded as being sufficiently purified for practical use but it should not be assumed, however, that these were single cell cultures.

The methods employed in preparing the culture media, in staining, and in making biochemical tests were those found in the latest editions of the *Manual*

TABLE I

DATA RESPECTING THE BACTERIAL PLANT PATHOGENS USED AS TEST ORGANISMS IN THIS INVESTIGATION

Identification no. used in experiments	Name of organism*	Disease caused	Host	Locality	Date and by whom isolated**
1	*Pseudomonas glycinea* (No. 1636)	Bacterial blight of soybean	Soybean leaf	Winnipeg, Manitoba	Aug. 1943, W. A. F. Hagborg
	P. glycinea (P.g. 1)	"	"	Vineland, Ontario	Aug. 1951, Z. Patrick
2	*P. lachrymans* (No. 3416)	Angular leafspot of cucumber	Cucumber leaf	Dauphin, Manitoba	July 1948, W. A. F. H.
	P. lachrymans (P.l. 2)	"	"	Vineland, Ontario	Aug. 1951, Z. P.
3	*P. coronafaciens* (No. 3003)	Halo blight of oats	Anthony oats	Winnipeg, Manitoba	June 1939, W. A. F. H.
	P. syringae (No. 2009)	Lilac bacterial blight	Nokomis lilac twig	Morden, Manitoba	June 1945, W.A.F.H.
4	*P. syringae* (P.s. 20)	"	"	New York State	1946, W. H. Burkholder
	P. syringae (P.s. 4)	(Bacterial leafspot of beans)	Bush kidney bean leaf	Vineland, Ontario	Aug. 1951, Z.P.
5	*P. pisi* (No. 3211)	Bacterial blight of peas	Dashaway peas	Portage La Prairie, Manitoba	Sept. 1946, W.A.F.H.
	P. pisi (P. s. 5)	"	Pea leaf	Vineland, Ontario	June 1951, Z.P.
6	*P. phaseolicola* (No. 3574)	Halo blight of beans	Snap beans	Transcoma, Manitoba	July 1949, W.A.F.H.
	P. phaseolicola (P. p. 6)	"	Lima bean leaf	Vineland, Ontario	Aug. 1951 Z.P.
7	*Xanthomonas campestris* (No. 3623)	Cabbage black-rot	Cabbage leaf	St. Vital, Manitoba	July 1948, W.A.F.H.
8	*X. pruni* (No. 3632)	Bacterial spot of peaches	Peach leaf		Dec. 1949, H. H. Thornberry (through W.A.F.H.)
	X. pruni (X. p. 8)	"	"	Vineland, Ontario	Aug. 1951, Z.P.
9	*X. translucens* f. sp. *cerealis* (No. 3042)	(Bacterial black-chaff of wheat)	Thatcher ×44.7 wheat	Winnipeg, Manitoba	Aug. 1943, W.A.F.H.
	X. translucens f. sp. *cerealis* (x.t. 9v) (x.t. 9G)	Bacterial stripe of barley, wheat, and rye	*Lolium multiflorum* blade	Vineland, Ontario	July, 1951, Z.P.

* *The bracketed letters and numbers following the names of each organism are the reference numbers to stock cultures of the original isolates.*

** *The writer is indebted to Dr. W. H. Burkholder and Dr. W. A. F. Hagborg for supplying some of the isolates.*

TABLE I—*Continued*

DATA RESPECTING THE BACTERIAL PLANT PATHOGENS USED AS TEST ORGANISMS IN THIS INVESTIGATION—*Continued*

Identification no. used in experiments	Name of organism*	Disease caused	Host	Locality	Date and by whom isolated**
10	*Corynebacterium flaccumfaciens* (No. 3291)	Bean bacterial wilt	Navy bean seed	Chatham, Ontario	June 1947, W.A.F.H.
	C. flaccumfaciens (C.f. 10)	"	"	Holland	1929, W.H.B.
	C. flaccumfaciens				Am. type culture collection (through W.H.B.)
11	*Erwinia amylovora* (E. a. 11p)	Fire blight	Pear twig	Vineland, Ontario	Sept. 1951, Z.P.
	E. amylovora (E. a. 11a)	"	Apple twig	Vineland, Ontario	Sept. 1951, Z.P.
12	*X. juglandis* (x.j. 12)	Walnut blight	English walnut twig and leaf	Vineland, Ontario	July 1951, Z.P.
13	*Agrobacterium tumefaciens*	Crown gall		University of Toronto	
	A. tumefaciens	"	Raspberry root gall	Vineland, Ontario	Sept. 1951, Z.P.
14	*E. tracheiphila*	Bacterial wilt of cumcumber	Cucumber	New York State	Sept. 1951, W.H.B.
16	*Bacterium stewartii* (S.S. 12)	Stewart's disease of corn	Corn	New York State	1941, W.H.B.
17	*P. marginata* (P.m. 17)	Gladiolus scab	Gladiolus leaf	Vineland, Ontario	Aug. 1951, Z.P.
17B	*P. marginata* (?)		Gladiolus leaf	Vineland, Ontario	Aug. 1951, Z.P.
18	*X. corylina* (x.c. 18)	Bacterial blight of filbert	Filbert twig	Vineland, Ontario	Sept. 1951, Z.P.
19	*X. pelargonii* (x. p. 19)	Leafspot of geranium	House geranium leaf	Toronto, Ontario	Oct. 1951, Z.P.
20	*X. carotae* (X.c. 20)	Carrot bacterial blight	Carrot leaves	Vineland, Ontario	Sept. 1951, Z.P.
21	*P. woodsii* (P.w. 21)	Bacterial leaf spot of carnations	Carnation leaf	Vineland, Ontario	Sept. 1951, Z.P.
24	*E. atroseptica* (?) (E.C. 24)	Black-leg of potatoes	Cabbage leaf	Vineland, Ontario	Sept. 1951, Z.P.
E 24	*E. atroseptica* (E. 24)	Black-leg of potatoes	Potato tuber	New York State	1945, W. L. Smith Jr. (through W.H.B.)
E 25	*E. atroseptica* (E. 25)	"	"	Florida	1946, W.H.B.
M.N.	*E. atroseptica* (M.N.)	"		Edinburgh, Scotland	1951, N. Noble (through W.H.B.)

TABLE I—*Concluded*

DATA RESPECTING THE BACTERIAL PLANT PATHOGENS USED AS TEST ORGANISMS IN THIS INVESTIGATION—*Concluded*

Identification no. used in experiments	Name of organism*	Disease caused	Host	Locality	Date and by whom isolated**
E 30	*E. carotovora* (E. 30)	Soft rot	Iris leaf	New York State	1945, W.H.B.
C 3	*E. carotovora* (C. 3)	"	Celery stalk	New York State	1944, W.H.B.
E 9	*E. carotovora*	"	Lettuce	New York State	1945, W.H.B.
24 V	*E. carotovora* (?)	"	Cabbage leaf	Vineland, Ontario	Aug. 1951, Z.P.

of Methods for Pure Culture Study of Bacteria (28) and in the *Difco Manual of Dehydrated Culture Media and Reagents* (11). Where a deviation occurs as to the composition of the media, etc., it is so stated.

Several pathogenicity tests were carried out for most of the isolates, usually on the same variety of plant from which the pathogen was isolated. The methods employed for inoculating the host plant were similar to those described by Riker *et al.* (34).

Media Used for Growing Plant Pathogenic Bacteria

It has been observed by Smith (37) and many other workers that attenuation of isolates may occur after successive transfers and the virulence of many plant pathogens declines and is lost when they are maintained in culture for any length of time. If one consults the literature further it is noted also that many bacterial plant pathogens will remain virulent in culture for some time if certain nutritional requirements, which are somewhat different for each genus, are satisfied (8). It was shown (8, 9), for example, that while certain plant pathogenic species in the genus *Pseudomonas* will lose their virulence if maintained on a medium rich in dextrose (8, 39), those in the genus *Xanthomonas* lose their virulence in a medium poor in dextrose. To satisfy these and other nutritional requirements and yet avoid making the medium too complex, one having the following composition was used for growing species from the genera *Pseudomonas*, *Erwinia*, *Agrobacterium*, *Corynebacterium*, and *Bacterium*:

Bacto-peptone	5 gm.
Beef extract	3 gm.
Dextrose	2 gm.
Yeast extract	5 gm.
Sodium chloride	2 gm.
Potassium phosphate	1 gm.
Agar	17 gm.
Tap water	1000 ml.
pH 6.6–6.8	

The nutrient broth used was the same as above but free from agar. For species in the *Xanthomonas* group, which require a medium rich in sugar, 20 gm. of dextrose was added to the above-mentioned medium.

Stock Cultures

Organisms to be used for immediate experiments were grown on the appropriate media mentioned above and transferred to fresh slants every two weeks. To keep the cultures as free as possible from cultural changes often produced by the conditions of cultivation and environment, and to slow down as much as possible the production of variants through mutations, stock cultures were maintained in duplicate. One set was maintained in tubes of sterile soil (17, 31) kept in the refrigerator at 5° C. and the other on nutrient agar slants covered with a layer of sterile mineral oil (25, 33, 36).

To avoid the possibility of obtaining variations in experimental results through the accumulation of resistant forms of the test organism or other changes which often occur when organisms are subcultured continuously, bacteria taken from stock cultures were used for each new set of experiments and they were not kept longer than for eight subcultures. To guard against the possibility of avirulent organisms being used as test organisms, pathogenicity tests were carried out with most of the cultures before the experiments and after they were completed.

B. Soils Used for the Isolation of Antagonistic Microorganisms

Although it has been established that certain common species of microorganisms are found fairly constantly in soils and the microflora and microfauna of the soil are broadly characteristic (4), it is also well known that different crops and different cultural practices may modify considerably the qualitative types and quantitative proportions of microorganisms present in a soil (38, 40, 42). To get the characteristic soil association and to avoid selective crop effects as much as possible, a "virgin" type of soil was used in most cases. By virgin is meant a soil type which was not planted to crops for at least ten years. Soil samples were taken up to ten inches in depth and the platings were done as soon as possible, and not longer than two weeks after collecting, the soil in the meantime being kept moist at 10°–15° C.

Soils of the following types and from the localities indicated were used:

Meadow soil; clay loam; Nashville, Ont., collected in May.

Virgin field soil; clay loam; Nashville, Ont., collected in Sept.

Sugar beet root-rot soil; clay loam; Harrow, Ont., collected in May.

Sugar beet root-rot soil; clay loam; Harrow, Ont., collected in Oct.

Forest soil; Nashville, Ont., collected in Oct.

Field soil; sandy loam; Camp Borden, Ont., collected in July.

Vineland fine sandy loam (planted to sod alfalfa, blue grass, and other green manure crops for 15 years); Vineland, Ont., collected in Sept.

Vineland clay loam; Vineland, Ont., collected in Sept.

Muck soil; Mt. Albert, Ont., collected in Oct.

C. Methods of Isolating Antagonistic Microorganisms

Several methods, none of which is ideal, are available for the isolation of antagonistic microorganisms from the soil. One which gave consistent results and which was used throughout these experiments is a modification of Waksman's "bacterial agar plate method" (41, 42, 43, 44). In this method a susceptible test organism is necessary as the indicator of antagonism. The comparative potency or the amount of the antibiotic products elaborated by the antagonist and the susceptibility of the test organism to these products is roughly indicated by the width of the resulting inhibition zones within which no growth of the test organism occurs. Organisms differ in their susceptibility to different antibiotic substances, and therefore the type of test organism used for the detection of antagonists influences greatly the numbers and types of antagonistic organisms that will be observed when dilutions of various soil samples are plated out. In this study different species of plant pathogenic bacteria served as the test organisms. The plant pathogen for which antagonists were being sought was incorporated into a suitable agar medium at 42° C. and 20-ml. portions poured into a series of Petri plates containing 1-ml. amounts of the various soil samples which had been diluted from 1 : 100 to 1 : 100,000 times with sterile water (17). The contents of the plates were mixed and incubated at 25°–28° C. After from 1 to 10 days' incubation the presence of antagonists was manifested by the formation of clear inhibition zones surrounding certain of the colonies. In these areas the test organism had made very little or no growth. The organisms producing these clear inhibition zones were isolated and tested individually for antagonistic properties.

To see if there were in the various soil samples significant differences in the relative numbers of organisms which were capable of exhibiting antagonism in culture against the different bacterial plant pathogens, the number of antibiotic producing organisms that were observed when one plant pathogenic species was used as the test organism was compared with the number of antagonists observed when other pathogenic species were the test organisms. In this way, by using as test organisms species from each of the genera that contain bacterial plant pathogens, the relative abundance of organisms antagonistic to each of the pathogens was determined.

Media Used for Detecting Antagonistic Microorganisms

The following media were used for the detection of antagonistic organisms:

(*A*) Nutrient agar (low in dextrose)

Bacto-peptone	5 gm.
Beef extract	3 gm.
Dextrose	3 gm.
Potassium phosphate	1 gm.
Sodium chloride	2 gm.
Yeast extract	1 gm.
Agar	17 gm.
Water	1000 ml.
pH 6.6	

(*B*) Nutrient agar (high in dextrose)
12 gm. of dextrose added to medium *A*.

(*C*) Potato-dextrose agar

(*D*) Soil-extract agar
Both media "*C*" and "*D*" were prepared according to the formulae found in Fred and Waksman's Laboratory Manual (17).

(*E*) Malt-nutrient agar

Malt	15 gm.
Bacto-peptone	3 gm.
Beef extract	2 gm.
Potassium phosphate	1 gm.
Agar	17 gm.
Water	1000 ml.
pH 6.4	

Preparation of Test Organisms for Seeding the Agar Media

The plant pathogens to be used for seeding into the various agar media were grown at 28° C. for 24 hr., or for 48 hr. for slow-growing forms, in a nutrient broth medium, and 5-ml. portions of these broth cultures were used for seeding into 330 ml. of cooled agar media.

In order to make comparisons possible, one species from each genus was used as the test organism in each series of platings of each soil sample, except in the two largest genera, *Pseudomonas* and *Xanthomonas*, where two species were used.

Isolation Procedures

The appropriate media were prepared the day they were to be used and were kept from solidifying in a water bath held at 50° C. Soil dilutions, from 1 : 100 to 1 : 100,000, were prepared according to the method described by Fred and Waksman (17) and 1-ml. portions of the various soil dilutions were pipetted into sterile Petri dishes. The dishes were prepared in duplicate, two of each dilution for each of the media used. Then 5-ml. portions of a 24–48 hr. broth culture of the required test organism were added to the flasks of cooled media, mixed thoroughly, and approximately 20 ml. poured into the Petri plates containing the series of soil dilutions. The contents of the Petri dishes were mixed thoroughly and quickly before the agar hardened. The plates were inverted and incubated at 27° C. They were examined after two days and those with the lower dilutions (1 : 100 and 1 : 1000) had many colonies developing on them, some of which displayed inhibition zones. The inhibition zones were counted and some of the strongly antagonistic organisms were isolated. The plates with the higher dilutions were allowed to incubate longer and were examined after from 6 to 10 days. The inhibition zones were counted and the more active antagonists were isolated. The antagonists were first isolated and grown on slants of the same medium on which they appeared originally. The antagonistic effect of the various microorganisms was then retested in pure culture following the methods described by Wilkins (47).

The selected antagonists were inoculated into tubes of moist sterilized soil so that their cultural characteristics would be kept as constant as possible (42).

D. Method of Testing the Antibiotic Action of the Isolated Antagonists

Using representative species of plant pathogenic bacteria from each genus as test organisms, a number of antagonists were isolated from the various soils mentioned. A preliminary screening test was first carried out to see whether each antagonist, tested individually, would cause inhibition of the test organism that was used in isolating it, as well as to determine the intensity of the antagonistic effect exerted on the test organism in culture and to see if any of the antagonists had lost the ability to produce inhibition zones after isolation. The type of medium used for testing the intensity of antibiosis was the same as that originally used for the detection and isolation of the antagonists. The antibiotic activities of the isolated microbes were tested by the simultaneous inoculation of the antagonist and test organism into the appropriate agar medium (41, 42, 47). For the test, a 24–48 hr. broth culture of the test organism was incorporated into a suitable agar medium, 5 ml. of broth suspension to 330 ml. of cooled media. This bulk seeded agar suspension was poured into sterile Petri plates at the rate of 18 ml. per plate. When the seeded agar solidified, a 3-mm. strip from a seven-day-old culture of an antagonistic colony was placed on the surface of the seeded agar. The plates were incubated at 25°–27° C. and inhibition zones measured after 48, 96, 150, 200, and 300 hr. of incubation. The degree of antibacterial activity was recorded as the distance in millimeters from the edge of the growth of the antagonist to the point where growth of the test organism was apparent, as is indicated by the arrow in Plate I, Fig. 1.

After this preliminary screening test, 120 of the antagonists which gave the largest inhibition zones were selected and their "bacteriostatic spectrum" against 28 species of plant pathogenic bacteria was determined.

E. Methods of Testing the "Bacteriostatic Spectrum" of 120 Antagonistic Microorganisms

For comparable results the same type of medium was used in all the tests. The medium selected had to be one which was most suitable for the growth of the different genera and species of bacterial plant pathogens, as well as for the growth of the antagonists, and which did not impair their production of antibiotic substances. A number of media were tried, few of which gave satisfactory results. The medium finally adopted for this series of tests had the following composition:

Bacto-peptone	5 gm.	Postassium phosphate	½ gm.
Beef extract	3 gm.	Sodium chloride	½ gm.
Yeast extract	1 gm.	Agar	17 gm.
Malt	10 gm.	Water (tap)	1000 ml.
Dextrose	5 gm.	pH 6.6	

Two methods were adopted for determining the bacteriostatic spectrum of the selected antagonists. One was the simultaneous inoculation of antagonist and

test organism as was described above. In this case, however, the antagonist was tested for its antibiotic activity not only against its specific test organism but also against the 27 other bacterial plant pathogens, and the relative susceptibility of each to the antibiotic action of the various antagonists was determined. The other method, which often gave more pronounced results, was the successive inoculation of the test organism after the antagonist had already made some growth. In this agar "cross-streak method", the antagonist was streaked in an even band across the top of the agar plate. After incubation for 24–48 hr. to allow the active substances to be produced and to diffuse out, the plant pathogenic bacteria, from 24-hr. broth cultures, were streaked at right angles to the antagonist. The antagonistic activity was shown as the distance between the antagonist and the beginning of growth of the bacterial pathogen. By this method it was possible to test all the plant pathogenic species of bacteria from one genus on a single plate and the relative susceptibility of each plant pathogen to the antibiotic substances produced by different antagonists was more readily apparent.

Observations and Results

A. Comparative Numbers of Soil Microorganisms Antagonistic to a Number of Phytopathogenic Bacteria

The numbers of microorganisms present in the various soils which were antagonistic to each of the plant pathogenic bacteria tested are summarized in Table II. The method used for the detection of antagonisms has been described in some detail under "Materials and Methods". It should be stressed, however, that the many studies made by others on microbial antibiosis indicate that the results may vary considerably depending on the method of testing, the condition of growth and stage of development of the antagonist, the concentration and stage of growth of the test organism, the composition of the media, and many other factors. Consequently, meaningful comparisons are often very difficult to make. So that some of the variables inherent in this type of culture experiment would be as constant as possible, the method adopted for the detection of microbial antagonisms was one that brought the two types of organisms dealt with—the plant pathogen and the antagonist—together simultaneously, so that one was not affected before the other by the accumulation in the culture medium of staling and other diffusable products. By the simultaneous inoculation into the appropriate medium of the plant pathogen—for which the number of antagonists is to be determined—and the fresh soil sample in various dilutions—with its antagonistic and nonantagonistic microflora—the two types are brought together in the form of isolated cells, spores, mycelial fragments, etc., in a fresh environment with a negligible concentration of their metabolic products. By this method, it was thought that only those organisms which can produce their inhibiting effects very rapidly, through the production of antibiotics or some other form of antagonism, that is, organisms having an "inherent property" for antibiosis, would show inhibition zones around their colonies. It has been observed by

TABLE II

SUMMARY OF THE COMPARATIVE NUMBERS OF MICROORGANISMS FOUND IN THE VARIOUS SOILS ANTAGONISTIC TO EACH OF THE PLANT PATHOGENIC BACTERIA TESTED

Test organism	No. of platings made	No. of antagonists observed	Frequency index $\left\{\frac{\text{No. of antag.}}{\text{No. of platings}}\right\}$	Antagonists isolated	
				Total No.	% producing large inhibition zones
1—*P. glycinea*	35	118	3.37	56	14
2—*P. lachrymans*	28	31	1.1	28	7
3—*P. coronafaciens*	39	106	2.7	60	10
4—*P. syringae*	45	81	1.8	35	6
5—*P. pisi*	21	44	2.1	44	11
6—*P. phaseolicola*	28	84	3.0	45	9
17—*P. marginata*	45	5	0.11	5	20
7—*X. campestris*	57	127	2.23	81	24
8—*X. pruni*	24	29	1.2	9	33
9—*X. translucens*	54	173	3.2	68	25
12—*X. juglandis*	31	55	1.78	29	31
18—*X. corylina*	31	37	1.2	20	35
19—*X. pelargonii*	5	7	1.4	7	30
20—*X. carotae*	6	11	1.83	11	36
11—*E. amylovora*	31	47	1.51	44	14
24*V*—*E. carotovora*	25	25	1.0	22	9
14—*E. tracheiphila*	10	44	4.4	28	61
13—*A. tumefaciens*	28	107	3.82	25	64
16—*B. stewartii*	32	24	0.75	20	10
10—*C. flaccumfaciens*	38	106	2.8	49	38

a number of investigators (21, 22, 23) that because of the very complex nature of the soil environment with the diverse activities of its microbiological populations, it is very difficult to build up in the soil high concentrations of certain antibiotic substances (29). It has also been reported that the potency of antibiotics may be markedly reduced because of the rapid breakdown of some by the action of soil organisms and inactivation of others, especially those with basic properties, through adsorption by the soil. Because of the operation of so many limiting factors it was felt that where the suppression of certain plant pathogens is due to the antagonistic action of some soil microorganisms and their antibiotic products, the major part of this suppression should be attributable to those antagonists that act rapidly. Such microbes should play the most significant role in the soil antagonisms. Possibly some potentially important antagonists were not shown by the technique used because they did not cause lysis of the test organism or their inhibiting substances took longer to form or to accumulate. This same technique, however, was used with all the pathogens, the same source of error applies to all and consequently the trends brought out by the survey should not be markedly affected.

The results in Table II show that with the techniques used over 1200 organisms were observed which were capable of inhibiting in culture the growth of the various bacterial plant pathogens. Some 686 of these were isolated for further tests, of which 401 were actinomycetes, 212 bacteria, and 73 fungi.

Of the antagonists isolated over 120 were found to produce very large inhibition zones when retested against their specific test organisms. When the intensity of the antibiotic effect exerted on the test organism by this group of antagonists was tested by the previously described simultaneous inoculation method the inhibition zones produced measured from 5 to 15 mm. in diameter. If these same antagonists were allowed 36 to 48 hr. growth before the specific test organisms were introduced into the plate as is done in the successive inoculation method usually used in testing the intensity of such antagonisms (27, 41), then most of them produced inhibition zones of from 20 to 45 mm. in diameter.

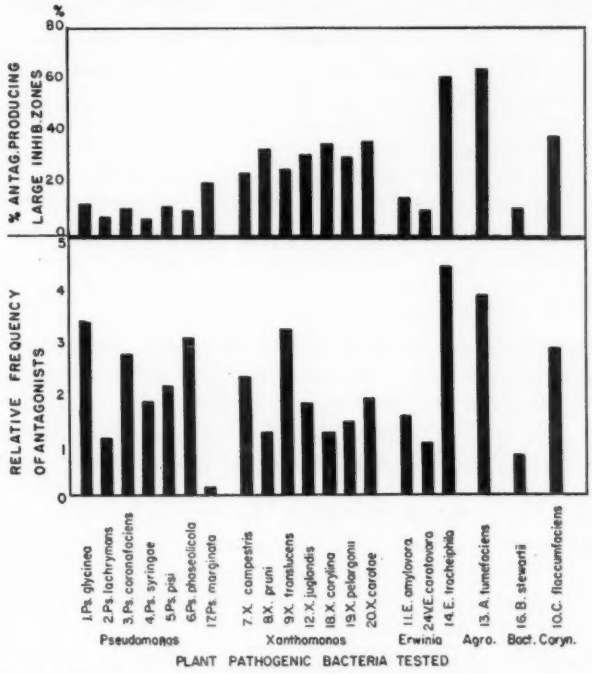
For convenience in the interpretation of the results a "relative frequency of antagonists index" was evolved which attempts to show, on a relative basis, the number of antagonistic organisms found in the various soils against each of the plant pathogenic bacteria tested. The "relative frequency index" was obtained by dividing the number of antagonists found for each plant pathogen by the number of times the pathogen was used as test organism. The frequency indices thus obtained for the various pathogens are shown in the lower graph of Text-Fig. 1, the upper graph of which shows the percentage of the antagonists isolated which, when retested against their specific test organisms, produced large inhibition zones.

The results presented in Table II and in Text-Fig. 1 indicate that there are great differences in the numbers of antagonistic organisms present in the soil with respect to the different plant pathogenic bacteria tested. *Erwinia tracheiphila*, for example, has a "relative frequency index" of 4.4 indicating that many members of the soil microflora are capable of developing some form of antagonism against this pathogen. Moreover, 61% of all the antagonists isolated for this species produced very large inhibition zones when retested against the pathogen, so that *E. tracheiphila* appears to be quite susceptible to the antagonistic action of the soil microflora. *Pseudomonas marginata*, on the other hand, has a "relative frequency index" of only 0.11, which suggests that it is susceptible to the antagonistic effects of relatively few soil organisms and, indeed, only five antagonists were found for this pathogen in the 45 times that it was used as test organism.

Inhibiting Effects of P. marginata in Culture

When *P. marginata* was used as test organism to determine the number of antagonistic organisms effective against it in the soils, it was observed to inhibit very rapidly most of the soil microflora in the culture medium. Even in the plates with the lowest soil dilutions (1 : 100) very few organisms developed, whereas dilution plates prepared at the same time but having other plant pathogens as test organisms were completely overgrown with many soil saprophytes. The inhibiting substances produced by *P. marginata* seemed to

have a suppressing rather than a lethal effect on many of the soil organisms inasmuch as a great deal of the suppressing activity was lost after 10 days' incubation at 28° C. following which many organisms came up on the soil



TEXT-FIG. 1. Relative abundance in the soil of microorganisms antagonistic to the various bacterial plant pathogens. The lower graph shows the relative numbers of antagonists for each plant pathogen per plate that were found in the soil. The upper graph shows the percentage of the antagonists isolated which produced large inhibition zones.

dilution plates. It was further observed that *P. marginata* did not form such inhibiting substances when grown on a medium with a very low nutrient content. When broth cultures of *P. marginata* were incorporated into an agar medium composed of 17 gm. agar and 2 gm. potassium phosphate per liter of tap water and the medium was used for plating out the soil samples by the same methods described previously, the results obtained were quite different from those in which a nutrient agar medium was used. In this case many organisms came up in all the soil dilution plates, with a large proportion of the colonies showing inhibition zones. These results are summarized in Table III.

Some of the organisms which produced inhibition zones on the plain agar medium were isolated and retested for antagonistic properties on a nutrient agar medium, where all of them failed to produce inhibition zones. This

TABLE III

EFFECT OF MEDIUM AND INCUBATION TIME ON THE INTERACTION OF *P. marginata* WITH OTHER SOIL ORGANISMS

Incubation time (days)	*P. marginata* incorporated into:			
	Nutrient agar		Plain agar	
	Number of organisms		Number of organisms	
	Total observed	Showing inhibition zones	Total observed	Showing inhibition zones
7	0	0	Over 100	46
14	40	5	Over 100	69

suggests that there are probably many additional organisms capable of developing at the expense of the pathogen and acting as antagonists in situations where the nutrient substrate is limiting.

The inhibiting substances produced by *P. marginata* not only suppressed the growth of many soil saprophytes but were also very effective against many plant pathogenic bacteria. When small agar disks were cut from a nutrient agar culture of *P. marginata* and tested for their antibiotic activity against some of the other plant pathogens, inhibition zones were produced in plate cultures of *P. glycinea*, *P. coronafaciens*, *P. syringae*, *P. phaseolicola*, *C. flaccumfaciens*, *E. amylovora*, *X. juglandis*, and *X. translucens*. The susceptibility of the other plant pathogens was not tested.

While it would be unsafe to draw too general conclusions from the results presented in Table II and in Text-Fig. 1 the indications certainly are that there are present in the soil flora a great abundance of microorganisms intrinsically capable of antagonizing all but a few of the bacterial plant pathogens. There were great differences however in the numbers of antagonists found against different species, sometimes even within the same genera, and these differences may have some relation to the relative ability of these species to survive in the soil for different periods of time.

Judging from the "relative frequency of antagonists index" (Text-Fig. 1) *E. tracheiphila*, *A. tumefaciens*, *X. translucens*, *C. flaccumfaciens*, *P. glycinea*, and *P. phaseolicola* appear to be especially vulnerable to the deleterious effects of the antagonistic soil microflora. For the most part, however, species in the *Xanthomonas* group appear to be most sensitive to the antagonistic soil microflora. The largest number of antagonists were found for this group and, as shown in Table II and the upper graph in Text-Fig. 1, from 25 to 35% of these antagonists produced large inhibition zones when tested against this group of pathogens. Some species in the *Pseudomonas* group have many antagonistic organisms, but very few of these antagonists, from 6 to 15%,

have shown intense antibiotic action. These same antagonists, as will be shown later, have very intense antibiotic activity when tested against species in the *Xanthomonas* group.

Erwinia tracheiphila, *Xanthomonas translucens*, and *Agrobacterium tumefaciens* appear to be the most susceptible of the plant pathogens tested. Many organisms present in the soil produce substances to which these bacteria are very susceptible. Other investigators have shown (6) that *E. tracheiphila* overwinters in the striped cucumber beetle and if its insect vector is eliminated the disease will not be present. Results obtained in this study suggest that the insect, while serving as a vector for the pathogen, may also serve as a means of protection from the antagonistic soil microflora to whose action the pathogen is very susceptible.

Recent work has shown that *X. translucens* (2) can persist from one season to the next on seed, straw, winter hosts, such as winter wheat, quack grass, and brome grass, but does not survive in nonsterile soil for any appreciable length of time unless in association with plant parts. It was further shown that the number of viable bacteria in straw was considerably reduced when the infested straw was mixed with soil. The evidence here presented suggests quite strongly that the failure of this organism to persist among soil saprophytes may be causally related to the large number of antagonists effective against it.

A. tumefaciens, the crown gall organism, seems to be an exception. Organisms antagonistic to the crown gall bacterium are very abundant in the soil. Other investigators (1, 5, 10) have found that this pathogen is very susceptible also to the action of many antibiotics. Yet, it has often been stated (6) that this bacterium is indigenous to the soil and will persist there for long periods. A possible reason for this is offered. When the susceptibility of this organism to the inhibiting action of different antagonists was tested, it was often observed that on the periphery of the large inhibition zones produced by a number of the antagonists, there was an area of stimulated growth (see Plate I, Fig. 6). Thus there is a possibility that in a soil environment, under certain conditions, the concentration of the antibiotic might often be such as to cause a stimulation rather than an inhibition of growth of the bacterium.

Antagonists for *P. marginata*, *P. lachrymans*, and *P. syringae*; the soft rot species *Erwinia carotovora* and *E. atroseptica*; and *Bacterium stewartii* are relatively rare and it has been shown by other investigators (6) that, of these, *E. carotovora*, *E. atroseptica* (6), and *P. marginata* (30) are able to maintain themselves for some time without their host plant and to compete quite successfully with the soil microflora.

It is difficult to find in the literature pertinent data concerning the maintenance and survival in nonsterile soil of many of the plant pathogenic species of bacteria dealt with in this study. But a comparison of the results of those studies that have been made (2, 6) with the results of this investigation seems to indicate that there is a good correlation between the number of antagonists

against a pathogen and the ability of the pathogen to survive in the soil. It may be concluded, from the data presented, that the ability or inability of the various species of plant pathogenic bacteria to survive and remain virulent in a soil flora may be governed to a large extent by the number of antagonists present in the soil flora against that particular plant pathogen. Generally speaking, the likelihood of most plant pathogenic bacteria (with the exception of a few species) surviving in nonsterilized soil for any appreciable length of time is slight, and the probability of the soil serving as a habitat where the pathogenic species can persist from one season to the next and serve as a source of primary inoculum is, for most species, negligible. Most of the plant pathogenic bacteria survive in association with their host plants more or less protected from the antagonistic action of the soil microflora. They are able to survive because of their ability to remain alive in or on the seed, in cankers, blighted twigs, or galls, in living or dead leaves (6), straw, and other plant refuse (2), on wild hosts, in all of which the activity of antagonistic soil organisms is at a minimum.

B. Comparative Antagonistic Effects of 120 of the More Active Antagonists on 28 Species of Bacterial Plant Pathogens

The antibacterial action of 120 of the more active antagonistic organisms with respect to all 28 species of bacterial plant pathogens is summarized in Table IV. A basic difference should be noted between the results presented in Table II and Text-Fig. 1, and those in Table IV and Text-Figs. 2, 3, and 4. To obtain the results summarized in Table II, each plant pathogenic bacterium was brought into contact with the natural soil microflora obtained from suspensions of the various soils listed, and the number of organisms which developed some sort of visible inhibiting effect against that species was determined. At the same time a number of these specific antagonists were isolated and the intensity of the antibiotic effect exerted by each of them on its own specific plant pathogenic species was measured. Thus the results summarized in Table II and in Text-Fig. 1 give us a picture of the relative abundance of antagonistic organisms present among the soil microflora capable of antagonizing the various plant pathogenic species, and also of the relative proportions of these specific antagonists which produced intense antagonistic effects on their specific test organisms. Thus the antagonists were first selected on the basis of their antagonistic activity to a particular species of plant pathogenic bacteria, and to avoid confusion they will be called "specific antagonists" while the bacterial pathogens for which these specific antagonists were isolated will be called the "specific test organisms". This does not imply that all of these antagonists are specific in the sense that they affect only the plant pathogenic species which were used in their detection. Some of them, as will be shown later, will act upon a great number of plant pathogenic bacteria species, while others show greater specificity and affect only one or two species (see Text-Figs. 3 and 4).

From the data obtained with respect to the relative abundance of antagonistic organisms in the soil against each of the plant pathogenic species, it

TABLE IV

SUMMARY OF THE ANTIBACTERIAL ACTION OF 120 ANTAGONISTIC MICROORGANISMS ON 28 SPECIES OF BACTERIAL PLANT PATHOGENS

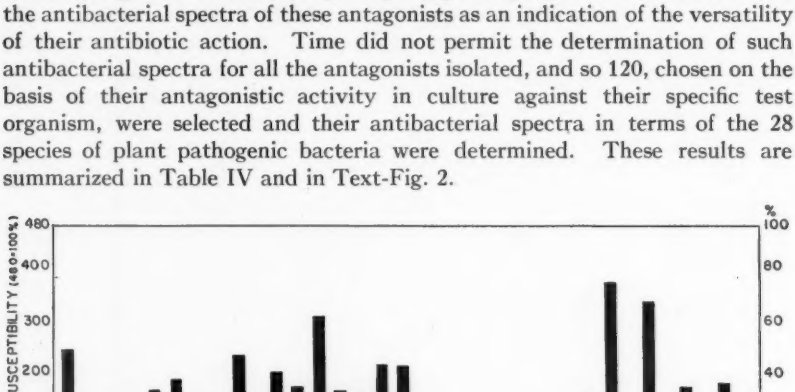
Test organism	Organisms inhibiting		Degree of antibiosis**						Relative intensity of antibiotic response*** (480 = 100%)	
	No.	%*	4	3	2	1	0	*S*	Total	%
1—*P. glycinea*	108	90	9	39	39	21	9	8	244	51
2—*P. lachrymans*	91	75	1	9	30	51	13	20	122	25
3—*P. coronafaciens*	93	78	0	7	33	53	23	4	136	28
4—*P. syringae*	87	71	0	8	27	52	32	2	128	27
5—*P. pisi*	99	81	2	17	37	43	20	6	170	35
6—*P. phaseolicola*	108	90	4	10	49	45	12	0	189	39
17—*P. marginata*	65	54	0	2	14	49	52	3	80	17
17*B*—*P. marginata* (?)	52	44	0	1	10	41	64	1	63	13
21—*P. woodsii*	115	91	4	27	54	30	4	1	234	49
7—*X. campestris*	114	95	1	18	52	43	6	4	201	42
8—*X. pruni*	109	90	4	11	45	49	9	1	187	39
9—*X. translucens*	117	97	24	46	35	12	2	8	308	64
12—*X. juglandis*	106	88	2	12	45	47	12	1	180	38
18—*X. corylina*	107	88	1	8	47	51	11	2	171	36
19—*X. pelargonii*	114	95	5	15	58	36	6	0	217	45
20—*X. carotae*	113	94	7	17	46	43	7	0	214	44
11—*E. amylovora*	90	75	1	16	31	42	26	4	152	32
14—*E. tracheiphila*	117	97	54	41	22	0	1	8	375	78
E24—*E. atroseptica*	62	50	0	6	27	29	60	10	91	19
E25—*E. atroseptica*	60	50	0	8	24	28	62	2	98	20
MN—*E. atroseptica*	65	51	0	11	26	28	58	7	106	22
24*V*—*E. carotovora* (?)	50	42	0	2	19	29	64	11	62	13
E30—*E. carotovora*	48	40	0	1	16	31	73	5	61	13
C3—*E. carotovora*	53	43	0	1	18	34	64	10	63	13
E9—*E. carotovora*	57	47	0	2	21	34	66	8	74	15
10—*C. flaccumfaciens*	94	79	3	20	45	26	9	7	181	38
13—*A. tumefaciens*	116	96	39	49	26	2	0	22	335	70
16—*B. stewartii*	96	80	2	16	45	33	20	5	174	36

* *Note: Percentage of the 120 antagonistic organisms giving measurable zones of inhibition.*

** Intensity rating		Zones obtained by "simultaneous inoculation"	Antagonist incubated 48 hr. before test organism added
4	=	Zone 9 mm. and greater	Zone 30 mm. and greater
3	=	Zone 4 to less than 9 mm.	Zone 20 to 29 mm.
2	=	Zone 0.5 to less than 4 mm.	Zone 10 to 19 mm.
1	=	Zone trace to less than 0.5 mm.	Zone trace to 9 mm.
0	=	No inhibition	No inhibition
S	=	Stimulation of growth (usually on the periphery of the inhibition zone).	

*** *Intensity of antibiosis rating (4, 3, 2, or 1; S = − 1) multiplied by the number of organisms in each of the columns, i.e. complete susceptibility to the 120 antagonists = 480 [120 (organisms) × 4 (highest intensity rating)].*

seemed of special interest to study the ability of these specific antagonists to inhibit the growth of the other plant pathogenic species; that is, to determine the antibacterial spectra of these antagonists as an indication of the versatility of their antibiotic action. Time did not permit the determination of such antibacterial spectra for all the antagonists isolated, and so 120, chosen on the basis of their antagonistic activity in culture against their specific test organism, were selected and their antibacterial spectra in terms of the 28 species of plant pathogenic bacteria were determined. These results are summarized in Table IV and in Text-Fig. 2.



TEXT-FIG. 2. Relative susceptibility of 28 bacterial plant pathogens to the antibiotic action of 120 antagonistic microorganisms isolated from the soil.

The intensity of the antibiotic effect exerted on the test organism was obtained by the two methods of determination discussed previously, the simultaneous inoculation of antagonist and test organism and the successive inoculation (26, 27) of the test organism after the antagonist had already made some growth. In most cases these two methods gave similar results but, as one might expect, in the successive inoculation method, which allows the antagonistic isolate 48 hr. growth before inoculating the test organism into the Petri plate, the inhibition zones produced were from two to five times greater than when both were inoculated into the plate simultaneously. Because of the variations which can occur in this type of experimentation, it was decided to use these two methods of testing in order that one might serve as a check on the other and where discrepancies occurred between the two, the experiments were repeated. On many occasions it was noted that the simultaneous inoculation technique was especially useful for the detection and demonstration of the lysis-causing properties exhibited by antibiotic substances produced by certain antagonists and was also good for demonstrating the stimulating

properties of the substances produced by other antagonists (see Plate I). The successive inoculation method, on the other hand, was especially useful for the detection of the highly specific antibiotic substances which were active only against certain plant pathogenic species (see Plate III, Figs. 15–24). In this case it was important to be certain that no matter how long the antagonist was grown, and hence no matter how high a concentration of the active substances was built up, it still would not affect other test organisms.

Antagonism of the test organism was first estimated by measuring the clear area between the antagonist and the plant pathogen as shown in Plate I, Fig. 1, and then, to check these results and also to get a more accurate estimate of the relative degree of susceptibility of each plant pathogen to the different antagonists, the various pathogenic species from each individual genus were tested on single Petri dishes as shown in Plate II. The distance from growth of the antagonist to the beginning of growth of a plant pathogenic bacterium, which was streaked at right angles to the antagonist, was taken as the zone of inhibition.

In the first two columns in Table IV, the number and the percentage of the 120 selected antagonists which inhibited the various plant pathogenic bacteria species are given. The degree of antagonism obtained by the two methods is indicated by the numbers 1 to 4 depending on the size of the inhibition zone produced, as is explained in the note at the end of Table IV. The antagonists which also caused a stimulation of growth of the test organism are listed under "*S*". To get some idea of the relative degree of inhibition exerted on each pathogenic bacterium by each of the 120 antagonists, each pathogen was rated for its "relative intensity of antibiotic response". The relative intensity rating was obtained by multiplying the "degree of antibiosis" rating (4, 3, 2, 1, and $S = -1$) by the number of organisms in each of the columns. Thus, on the basis of this rating an organism completely susceptible to all of the 120 antagonists would get a rating of 480, i.e. 120 antagonists $\times$ 4 (highest degree of antibiosis) and all the plant pathogenic species were compared to this.

The results summarized in Table IV and in Text-Fig. 2 indicate, as in the previous case, that there are considerable differences in the susceptibility of the various species of plant pathogenic bacteria to the action of antagonistic organisms. These results become much more significant when it is noted that the 120 antagonists used here were selected on the basis of their high antibiotic activity against their specific test organisms, and therefore all of them have quite intense antibiotic producing abilities. It is seen that 117 of the 120 selected antagonists, or 97% of them, are antagonistic to *E. tracheiphila* while only 48 or 40% act as antagonists to *E. carotovora*. Of the 117 antagonists of *E. tracheiphila* 95 produced relatively large inhibition zones (most of the *E. tracheiphila* streaks in Plate II failed to grow) whereas, of the 48 antagonists for *E. carotovora*, only one or two produced large inhibition zones and many of the *E. carotovora* streaks shown in Plate II were not at all affected by the antagonists. Inasmuch as each of the 120 antagonists was originally isolated from the soil, the determination of the comparative antibacterial effects of

these antagonists simply gives us an indication of the relative susceptibility of the various plant pathogenic species to a random number of soil antagonists. Consequently the results obtained in this set of experiments should to a large extent support those presented previously. Thus, plant pathogenic species for which the number of antagonistic organisms was relatively abundant should still stand out as the species affected by the largest number of the 120 selected antagonists, and the species for which the antagonists were relatively rare should be affected by the smallest number of these antagonists. It will be noted that the two sets of results do support each other rather closely in this respect.

Similarity of Reaction of Different Isolates of the Same Species of Plant Pathogenic Bacteria

There remains to be considered the consistency in the reaction of different isolates of the same plant pathogen obtained from different localities. To test the possibility that different isolates of the same species might produce different reactions, duplicate strains of *P. marginata*, three isolates of *E. atroseptica*, and four isolates of *E. carotovora* were used in all the experiments, and on many occasions different isolates of the other species were also tested against the same antagonist. The similarity of reaction which was obtained in all cases strongly suggests that the type of reaction obtained for one isolate will be identical with that of other isolates of the same species even from different locales (see Plate II, Figs. 7–10 and Figs. 11–14, and Plate III, Figs. 15–23). A similarity of reaction of duplicate strains of other plant pathogens has been

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FIGS. 1–6. Examples of a few types of antagonistic effects obtained when the test organism and the antagonist were inoculated simultaneously into the Petri dish.

FIG. 1. The test organism, *X. translucens*, was very susceptible to the antibiotic substances produced by the two *Streptomyces* isolates, hence the inhibition zones are large. Most of the *Xanthomonas* species in their antibiotic reactions produced such clearly and sharply delimited inhibition zones. The degree of antibiotic activity was recorded as the distance, in millimeters, from the edge of growth of the antagonist to a point where growth of the test organism was apparent, as is indicated by the arrow.

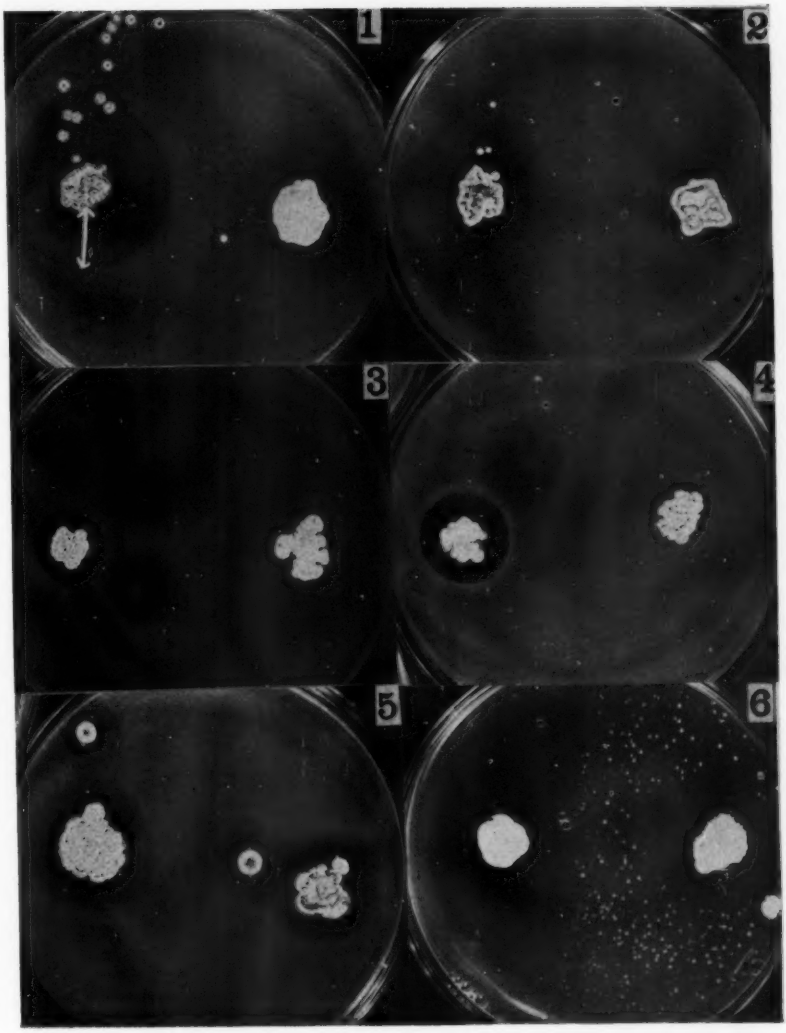
FIG. 2. This test organism, *E. carotovora*, was not noticeably inhibited by the antibiotic substances produced by the two antagonists, around which accordingly there are no inhibition zones.

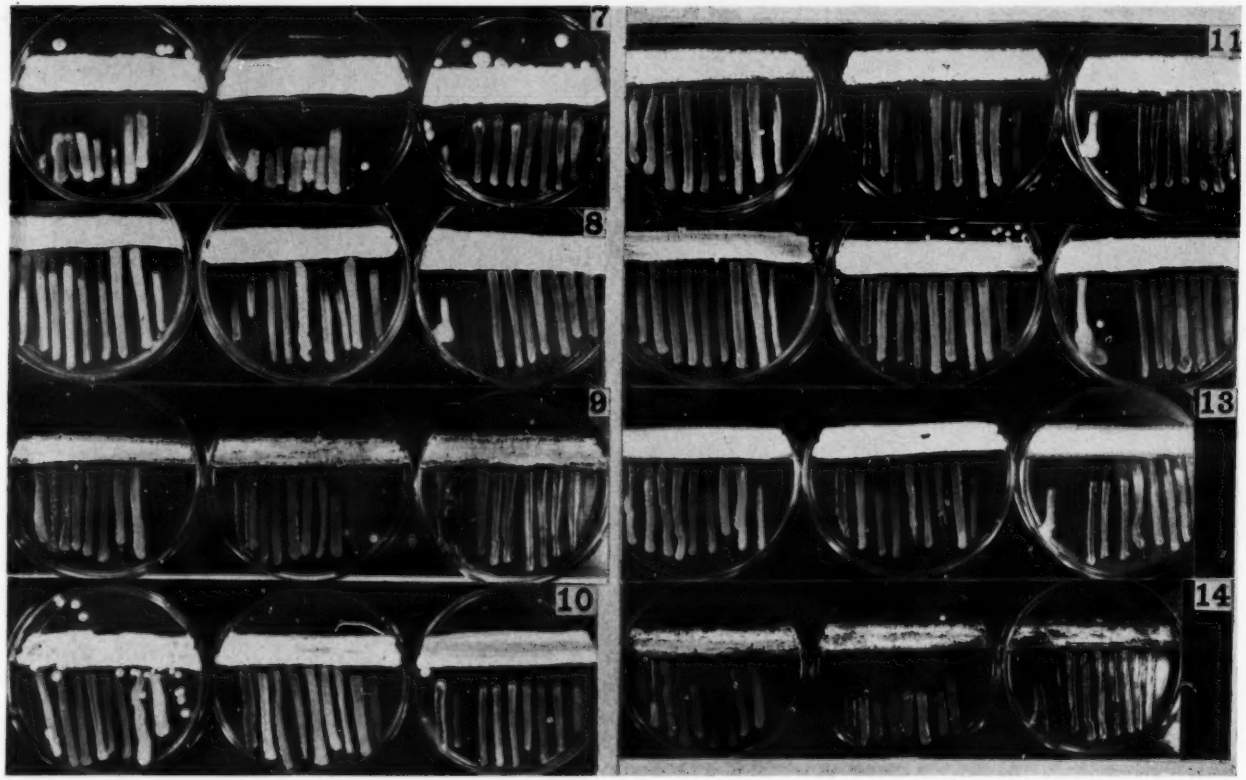
FIGS. 3 and 4. In a number of cases when *Corynebacterium flaccumfaciens* was tested it was observed that on the periphery of the sharply delimited inhibition zone the yellow pigment, which is characteristic of this pathogen in culture, became intensified and hence there would seem to have been some stimulation of growth of the pathogen.

FIG. 5. An example of the "target-ring" type of inhibition zone which was produced by a number of antagonists especially when tested against some *Pseudomonas* species. The explanation often given for these zoning effects is that many antagonistic organisms produce more than one type of antibiotic substance. Some of the substances are bacteriostatic, others bactericidal, and still others bacteriolytic, or one may have any combination of the three. Thus in an agar culture, because they have different diffusion rates or are produced at different times, they may become spacially separated and each will produce its own characteristic type of antagonistic effect in the areas that it has reached. It was also noted that while an antagonist will produce the "target ring" type of inhibition zones with one pathogenic species it may not do so with another, which suggests that some of the different toxic and lytic factors may be species specific.

FIG. 6. Shows a marked stimulating effect on the periphery of the inhibition zone which was obtained in this instance when *Agrobacterium tumefaciens* was the test organism.

PLATE I

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FIGS. 7–14. Examples of the relative sensitivities of the plant pathogenic bacteria to the antibiotic substances of eight different antagonistic isolates, as tested by the agar cross streak method. The antagonist is streaked in an even band across the top of the Petri dish 48 hr. before the bacterial plant pathogens are streaked at right angles to the antagonist. If species from the same genus are tested on the same Petri dish certain experimental errors can be eliminated. The plant pathogenic bacteria species are streaked in the same order in all cases, the order is as follows:—

The first Petri dish on the left contains from left to right the following *Pseudomonas* species:—

1—*P. glycinea;* 2—*P. lachrymans;* 3—*P. coronafaciens;* 4—*P. syringae;* 5—*P. pisi;* 6—*P. phaseolicola;* 17—*P. marginata;* 17*B*—*P. marginata* (?).

Petri dish in the center contains from left to right:—

7—*Xanthomonas campestris;* 8—*X. pruni;* 9—*X. translucens;* 12—*X. juglandis;* 18—*X. corylina;* 19—*X. pelargonii;* 20—*X. carotae;* 10—*Corynebacterium flaccumfaciens;* 16—*Bacterium stewartii.*

Petri dish on the right contains from left to right:—

11—*Erwinia amylovora;* 14—*E. tracheiphila;* 13—*Agrobacterium tumefaciens;* E24—*E. atroseptica;* E25—*E. atroseptica;* *MN*—*E. atroseptica;* E30—*E. carotovora;* *C3*—*E. carotovora;* E9—*E. carotovora;* 24*V*—*E. carotovora* (?).

FIG. 7. The antibiotic substances produced by this *Streptomyces* species inhibit, although to different degrees, most of the plant pathogens tested.

FIG. 8. The *Pseudomonas* species, except the two isolates of *P. marginata*, along with five *Xanthomonas* species were inhibited by the antibiotic substances of this *Streptomyces* isolate. None of the soft rot *Erwinia* species were inhibited.

FIG. 9. The antibiotic substances produced by this *Streptomyces* species were quite specific, affecting only the *Xanthomonas* species, *C. flaccumfaciens*, *E. tracheiphila*, and *A. tumefaciens*. The *Pseudomonas* and soft rot *Erwinia* species were not affected.

FIG. 10. All of the *Erwinia* species were inhibited by the antibiotic substances of this *Streptomyces* isolate.

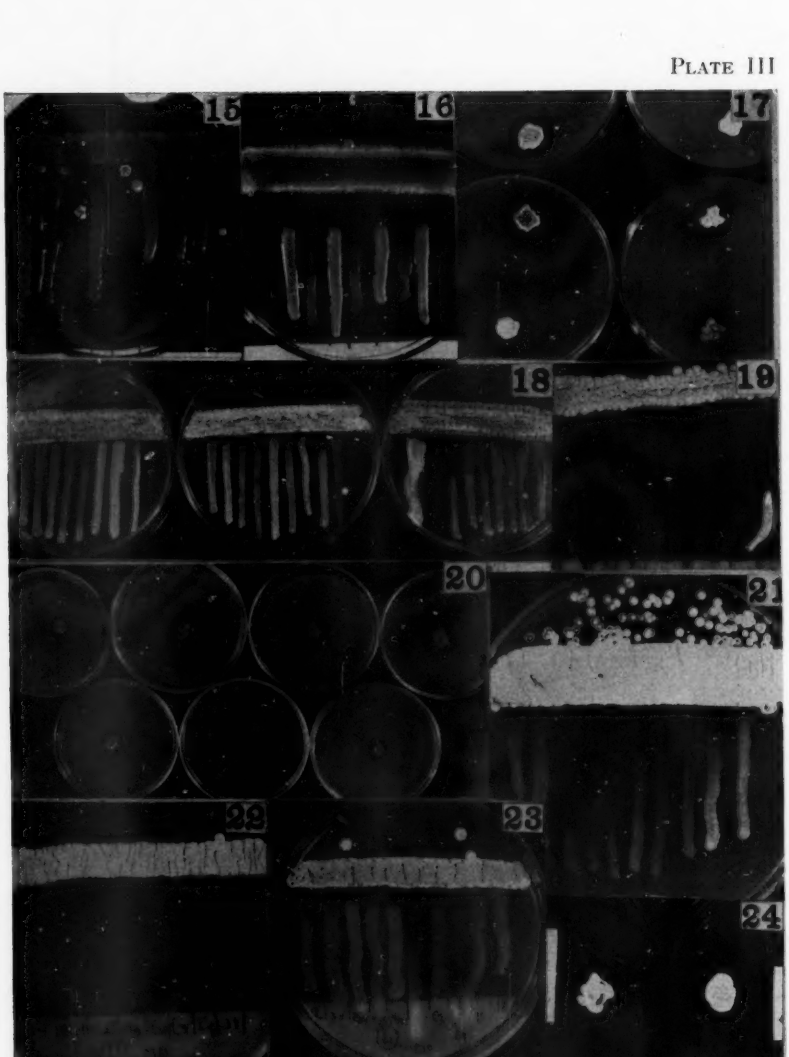
FIG. 11. Most of the pathogenic bacteria except *P. marginata* and *E. carotovora* were inhibited to some extent by the action of this antagonist.

FIG. 12 [below Fig. 11]. Only *P. phaseolicola*, *X. translucens*, *E. tracheiphila*, and *A. tumefaciens* were inhibited by this *Streptomyces* isolate.

FIG. 13. The antibiotic substances of this antagonist inhibited most strongly the *Erwinia* species.

FIG. 14. Although all the other plant pathogenic species were strongly inhibited by the antibiotic substances of this actinomycete the soft rot *Erwinia* species are not at all affected.

PLATE III



reported also by other investigators (27). Although it is known that some bacteria in constant association with an antibiotic will produce resistant forms (13), this is of little significance here since in all these experiments the pathogenic species was not reisolated after it had been allowed to react with the antagonist and so in each case we are dealing with what may be referred to as the "wild type" of organism.

A Graphic Representation of the Antibiotic Spectra of 36 Antagonistic Microorganisms Isolated from the Soil

A detailed antibiotic spectrum was determined for each of the 120 antagonistic organisms tested against all the available bacterial plant pathogens. To demonstrate some of the variations in susceptibility of each plant pathogen to the antibiotic substances of the various antagonistic isolates a more detailed graphic summary of the antibacterial spectra of 36 antagonistic isolates when tested against the various bacterial plant pathogens is presented in Text-Figs. 3 and 4. The relative sensitivity of each plant pathogen to the antibiotic action of individual antagonists, tested under identical conditions using common media, is shown. The most versatile antagonists which act upon the greatest number of plant pathogenic species and some of those which are more selective in their action can be readily picked out. The antagonistic organisms are listed in the vertical column on

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FIGS. 15–24. Illustrate the manner in which antibiotic reactions may be used in differentiating culturally and morphologically similar bacterial plant pathogens.

FIG. 15. The antagonist was streaked along the top of the Petri dish, *X. juglandis* and *X. corylina* were streaked alternately at right angles to the antagonist. *X. corylina* was not affected by the antibiotic substances produced, while *X. juglandis* was.

FIG. 16. *X. juglandis* and *X. corylina* were again streaked alternately. *X. juglandis* was more susceptible to the antibiotic substances produced than *X. corylina* and thus the two can be readily differentiated.

FIG. 17. Plates on the left contain *X. juglandis* as the test organism, those on the right contain *X. corylina*. Again *X. juglandis* was more susceptible to the antibiotic substances produced.

FIG. 18. Only *E. atroseptica* isolates were appreciably susceptible to the antibiotic products of this *Streptomyces* species.

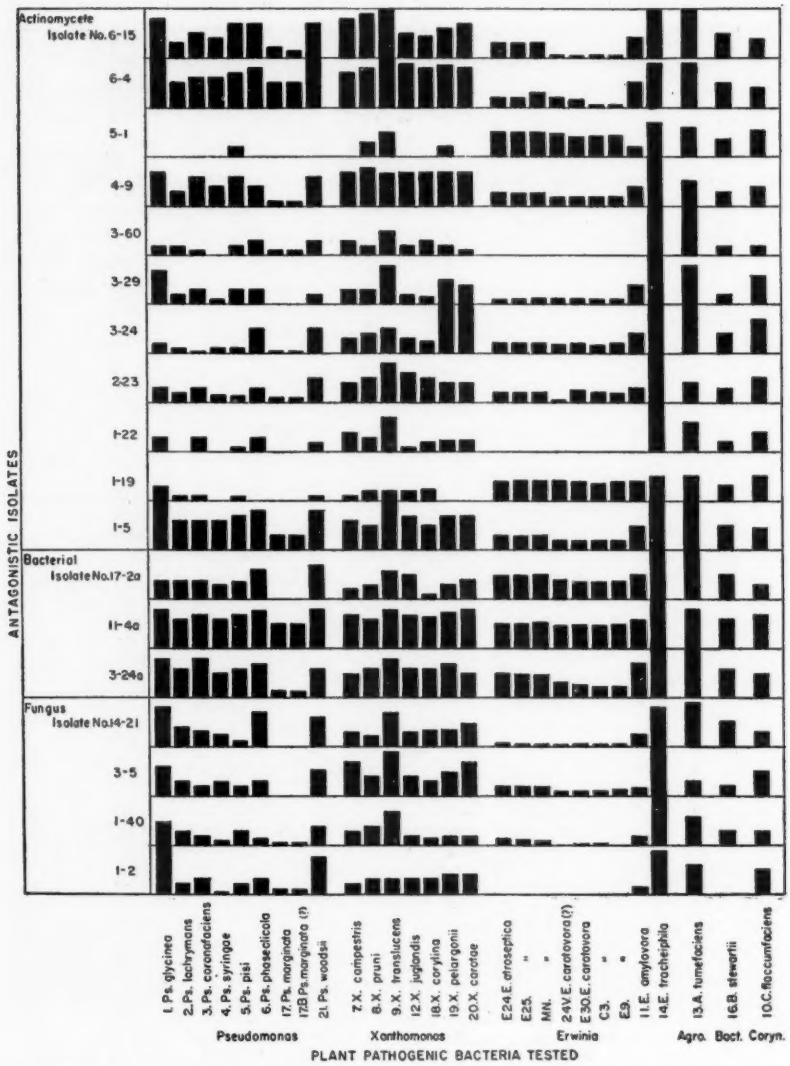
FIGS. 19, 21, 22. *E. atroseptica* isolates can be readily separated from the *E. carotovora* isolates on the basis of the degree of sensitivity to the antibiotic substances of a number of antagonists. In each case the streaks left to right are 24*V*–*E. carotovora* (?), three isolates of *E. atroseptica*, three isolates of *E. carotovora* (followed in some plates by other test organisms). In each case the *E. atroseptica* isolates are more strongly inhibited than are the *E. carotovora* isolates.

FIG. 20. The different susceptibilities of *E. atroseptica* and *E. carotovora* to the antibiotic action of the same antagonist when both the test organism and the antagonist were inoculated simultaneously into the Petri plate. The test organism in each Petri plate is:—top row, left to right, *E. carotovora* (?) (isolate 24*V*) followed by the three different isolates of *E. atroseptica* (in the order listed in Table I); bottom row, three different isolates of *E. carotovora*. The same antagonistic isolate was inoculated in the center of each of the seven Petri plates. In this instance as in the cases mentioned above, the *E. atroseptica* isolates were more strongly inhibited than were the *E. carotovora* isolates, hence the larger inhibition zones where *E. atroseptica* was the test organism.

FIG. 23. *E. atroseptica* and *E. carotovora* are equally affected by the antibiotic products.

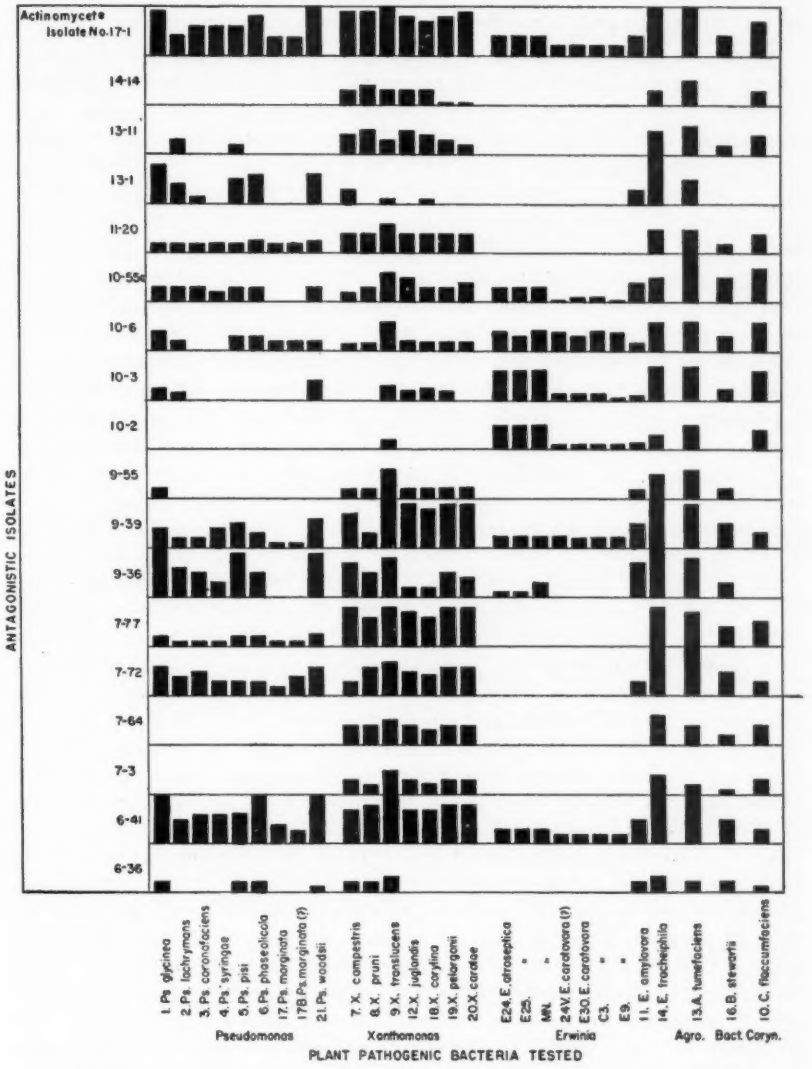
FIG. 24. *E. carotovora* is inhibited while *E. atroseptica* is stimulated by the substances produced by this *Streptomyces* isolate.

the left and the bacterial plant pathogens are listed along the bottom. The intensity of antibiotic effect exerted on the various pathogenic species by each antagonist is recorded as the height of the inked-in rectangles which



TEXT-FIG. 3. A graphic representation of the antibiotic spectra of 18 antagonistic soil microorganisms tested against 28 bacterial plant pathogens. The intensity of the antibiotic effect exerted on the various plant pathogens by each antagonistic isolate is recorded as the heights of the inked-in rectangles.

corresponds to the size of the inhibition zones produced. For example, since *P. glycinea* is very susceptible to the antibiotic substances produced by the fungus isolate No. 1-2, the rectangle which represents *P. glycinea* and the



TEXT-FIG. 4. A graphic representation of the antibiotic spectra of an additional 18 antagonistic microorganisms isolated from the soil tested against 28 bacterial plant pathogens.

antagonistic isolate 1-2 is completely inked-in. *P. lachrymans* on the other hand is only slightly susceptible to the products of the above-mentioned antagonist, hence the corresponding rectangle is only slightly inked-in, while *E. atroseptica* and *E. carotovora* are not susceptible to the antibiotic products of this antagonist and hence their corresponding rectangles are clear.

From the results presented in Text-Figs. 3 and 4 we can visualize more clearly the great differences in the susceptibilities of various plant pathogenic bacterial species to the antibiotic substances of individual antagonistic organisms. It will be seen that each antagonist is characterized by a specific antibacterial spectrum and that, while each produced antibiotic substances which are inhibitory to one bacterial species, only by specific tests can it be determined what other species will likewise find these substances inhibiting. Some antagonists produced antibiotic substances which acted upon a large number of plant pathogenic species, as for example actinomycete isolates Nos. 1-5, 6-4, and 6-15. These were found to be so highly effective against so many plant pathogenic species of bacteria that further study will be made with respect to the antibiotic substances they produce and the possibility of utilizing these in controlling some bacterial plant diseases. Other antagonistic organisms produced antibiotic substances to which certain groups of plant pathogens were especially susceptible. Cultures of actinomycete isolates Nos. 7-3, 7-64, 14-14, for example, produced substances to which the *Xanthomonas* species used in this study were very susceptible. Finally, to the antibiotic substances produced by *Streptomyces* cultures 10-2 and 10-3 only *E. atroseptica* appeared particularly sensitive. Since the antibiotic substances produced by these antagonistic organisms probably vary greatly in their chemical nature (15, 16) and in the manner in which they are produced, they would be expected to vary also in their action upon the cells of the different bacteria species. It is known that the bacterial cells vary greatly in their affinity for the different antibacterial agents (13) and susceptibility to these antibiotic agents reflects something of the biochemical and physiochemical organization of the bacterial cell (13, 15). Because of the remarkable plasticity of the bacterial cell with its ability to undergo reversible and irreversible modifications in its metabolic processes, it is realized that there is danger in trying to interpret some of the results obtained in terms of bacterial physiology and species relationships. Nevertheless the observed similarity in the reactions of some of the pathogenic species to the antibiotic action of some of the antagonists was interesting and surely not without significance. It was noted, for example, that *X. pruni*, *X. corylina*, and *X. juglandis*, species which affect trees, were inhibited by the same group of antagonists and to the same degree and only a few antagonists were specific for one and not for the other species. *X. carotae* and *X. pelargonii* appeared to make up another such group, separate from *X. campestris*. *X. translucens* also stands by itself, perhaps close to *X. carotae* and *X. pelargonii*, and it was highly susceptible to the antibiotic action of most of the antagonists. *Pseudomonas glycinea*, *P. phaseolicola*, and *P. woodsii* formed a very susceptible group in the genus *Pseudomonas* while *P. marginata*

was affected by most of the antagonists and with the same intensity as were the two soft rot species of *Erwinia*, *E. carotovora*, and *E. atroseptica*. Another interesting relationship was observed in the genus *Erwinia*. It was noted that *E. atroseptica* and *E. carotovora* have very different antibiotic spectra from *E. amylovora* and *E. tracheiphila*. The fact that the two latter species are susceptible to the antibiotic action of so many different antagonists as compared with the former tends to support other workers (6) in their separation of the soft rot species in the genus from those that produce blights in plants.

These and other similarities in reaction by the various pathogenic bacteria species to such a large number of antagonists must be of some significance. It may be that these plant pathogenic species have similar cellular constituents, structural or metabolic, and therefore species which are equally susceptible and react to the same degree with many of the different antibacterial products are somehow related to one another. By utilizing many antagonistic organisms with their great variety of chemical products (16) (toxic agents, specific lytic enzymes, vitamins, etc.), one could describe each bacterial species in terms of its reaction to different antagonists. By grouping the various species on this basis it is conceivable that, with the aid of other available characteristics, many of the uncertainties with respect to the relationships between groups of species within the same genus and perhaps relationships between different genera might be resolved.

A Method for Differentiating Culturally and Morphologically Similar Bacterial Plant Pathogens

If one consults *Bergey's Manual of Determinative Bacteriology* (3), it will be found that in many instances the descriptions of bacterial plant pathogens, especially those occurring in the genera *Pseudomonas* and *Xanthomonas*, are identical except for the characteristic of pathogenicity. It is difficult or impossible to identify some of them in the laboratory without going through many laborious biochemical or pathogenicity tests. Therefore other methods of differentiating even a few pathogens in culture would be useful. Two such species which are virtually identical culturally and morphologically and therefore very difficult to differentiate in culture are *X. juglandis* and *X. corylina* which cause respectively walnut blight and filbert bacteriosis (32). In some of our experiments it was observed that certain antagonistic organisms produced antibiotic substances which had differential effects on these two species. Thus the antagonist shown in Plate III, Fig. 15, produced antibiotic substances which did not seem to affect *X. corylina* but to which *X. juglandis* was quite sensitive. *X. juglandis* was also much more sensitive to the antibiotic substances produced by the antagonistic isolates shown in Plate III, Figs. 16 and 17. Thus it is possible to make use of specific antagonists to differentiate between two such species.

Similar difficulty is encountered in trying to separate the two soft-rot-producing species, *Erwinia atroseptica* and *E. carotovora* (7). Again a few antagonists were found to whose antibiotic products the two species had different susceptibilities. When the antagonist was streaked across the top

of the Petri dish and the three isolates of *E. atroseptica* and *E. carotovora* at right angles to the antagonist, the differences in susceptibilities show up very clearly, as seen in Plate III, Figs. 18, 19, 21, and 22. Other soft-rot-producing organisms were isolated (24 and 24*V*) but it was not known whether the isolates were *E. carotovora* or *E. atroseptica*. When the unknown was streaked alongside the two known species it was quite clear from Plate III, Figs. 18, 19, 20, 21, and 22 that the unknown species was *E. carotovora*. *E. atroseptica* was susceptible to the antibiotic substances of most of the antagonists, but this did not always hold true. In Plate III, Fig. 24, the antagonist causes stimulation of *E. atroseptica* isolates but produces inhibition zones with *E. carotovora*, while the antagonist in Plate III, Fig. 23, produces antibiotic substances to which both species appear to be equally susceptible.

To get best results in such tests it is important to streak the antagonist in an even band across the top of the Petri dish and to allow it to grow for 36 to 48 hr. Broth cultures of the pathogens in question should be of the same age, preferably 24 hr. old; the amount of inoculum is also important. One loopful should be used, and the streaks made in the same direction, towards or away from the antagonist, care being taken lest some spores from the antagonist be dragged by the loop and spoil the results. For clear-cut results it is best to read the plates after 20 to 30 hr. of incubation because after that time resistant forms tend to come up as shown in Plate III, Figs. 18 and 23.

It is possible likewise to find other antagonists whose antibiotic products are specific for other species (see Text-Figs. 3 and 4). Thus it may be possible to utilize the character of "specific antagonists" as an aid in differentiating many organisms in culture. With proper standardization and adaptation to the limitations, the method described above may serve as an additional means of separation and identification of bacterial plant pathogens.

Discussion and Summary

Since a limited number of plant pathogenic bacteria species and a relatively small number of soil types were dealt with, one cannot say to what extent the results obtained would be applicable to other bacterial species or other soil types. From the data here presented however, certain points of interest have been brought out and, although it would be unwise to generalize with respect to the possible significance of some of the results, certain trends have been noted and some cautious interpretations may be justified.

The results show that in their antibiotic reactions most of the plant pathogens behave as individuals rather than as generic groups; some are highly susceptible to the antibiotic products of many antagonists while others are relatively resistant to these substances. There are also great differences in the numbers of antagonists effective against different species, sometimes even in the same genus, that are present in a soil microflora. For most of the plant pathogens used in these experiments there are many antagonistic organisms present in the soil, while for a few such antagonists are relatively rare. The fact that so many antagonists have been found is interesting because most of

the plant pathogenic bacteria are Gram-negative and the better known antibiotic substances are relatively ineffective against the Gram-negative group (15).

Two methods were used for studying the relative numbers of organisms capable of antagonizing the different plant pathogens and the relative intensities of antibiotic effect exerted on each of them. One was the direct method in which specific plant pathogenic bacteria were used as test organisms in finding the number of antagonists that are present for each one in a soil sample. The other, the indirect method, used a number of antagonists isolated primarily on the basis of their antibiotic activity against specific plant pathogens and determined to how many antagonists, each of the other plant pathogenic species was susceptible, and to what degree. The results, which are summarized in Table II and in Text-Fig. 1, in Table IV and in Text-Figs. 2, 3, and 4, show that, with a few exceptions, no matter how the relative numbers and relative susceptibilities were determined those pathogenic species which had the largest number of antagonists as determined by one method were also the most antagonized group when tested against the random selection of antagonists; and the species for which antagonists were relatively rare were likewise least affected. This behavior may well reflect the relative ability of these species to survive in the soil for different periods of time. The *Xanthomonas* species as a whole are the most susceptible group (20). They are affected by most of the antagonists and to the highest degree. This is all the more significant when it is noted that the *Xanthomonas* group contains only plant pathogenic bacteria (6). Whereas the other plant pathogenic bacteria are fairly obviously related with various soil types, the *Xanthomonas* group, as far as is known, has no close counterpart among soil bacteria (6). Inasmuch as they are antagonized by a large group of soil saprophytes they seem to have lost the ability to live in the soil and are very dependent for their survival and activity on their host plants which buffer them from the antagonistic action of the soil flora.

Many of the plant pathogenic *Pseudomonas* species resemble green fluorescent soil types, and they are able to adapt themselves to live and compete with the other soil flora among which fewer organisms were found that were able to antagonize this group. In this genus however great variations were found from very susceptible species like *P. phaseolicola* to very resistant ones like *P. marginata*. This may correspond to the different stages of specialization in the evolution of a parasitic mode of life.

The genus *Erwinia* appears to be composed of two groups—the soft-rot-producing species, *E. carotovora* and *E. atroseptica*, which have very few antagonists in the soil flora and are therefore well adapted to live in the soil; and the other group, *E. amylovora* and *E. tracheiphila*, which produces blights in plants. The last two species are more specialized parasitically and hence less resistant to the action of soil antagonists. *E. tracheiphila* is more susceptible than *E. amylovora* and its possible dependence on an insect vector has been discussed.

Another point of interest brought out by the results shown in Text-Figs. 3 and 4 is that each antagonistic organism is characterized by a specific antibacterial spectrum, and those antagonists having the most intense antibiotic activity are, in general, also the most versatile in that they are capable of inhibiting the greatest number of bacterial species. Many antagonists, however, are more specific and still have intense antibiotic activity against specific types, and may well play an important role in the suppression of these types.

One of the more interesting results to come out of these investigations was the demonstration that the high specificity which characterizes some of the antibiotic reactions can be turned to good account in separating sharply, consistently, and with minimum effort such closely related species as *E. carotovora* and *E. atroseptica* or *X. corylina* and *X. juglandis*. The results have been so promising in this respect that the possibility and feasibility of characterizing bacterial plant pathogens in terms of antibiotic spectra should be further investigated.

It is very difficult to interpret some of these results and quite impossible to transpose them to a soil environment with its heterogeneous microflora and unlimited possibilities for interactions and associations. It is often very difficult or impossible to demonstrate the existence of antibiotics in a soil environment, and from this many conclude that antibiotics and antagonisms in the soil are of limited importance in the control of plant pathogens. It was shown, for example (29), that although a very high concentration of the purified antibiotics terramycin and aureomycin had to be added to the soil to inhibit *B. polymyxa* and *B. subtilis*, yet when *Streptomyces rimosus* and *S. aureofaciens*, organisms which produce these antibiotics, were added to the sterile soil, the sensitive organisms were inhibited. It is impossible to say whether or not the antibiotics are actually produced in the soil, since there is no satisfactory technique for their detection in low concentrations. It is known, however, that the capacity to produce antibiotics is particularly characteristic of microorganisms whose natural habitat is the soil (4). Therefore in many cases where antibiotics are said not to be produced, they may merely not accumulate and not be detectable in low concentrations. Even though for some of these antibiotics the rate of inactivation may be high in the soil, at the surface of the cells producing them a local concentration might be maintained which would be sufficient to have a marked biological effect on sensitive organisms in the immediate vicinity (4). Consequently it is the type of interaction encountered in such microenvironments that will be of decisive importance in determining the ability of any organism to survive in a soil environment. While these microenvironments will be functions of both the basic inorganic and the organic components of the soil in question, since the vast majority of the soil microorganisms are heterotrophic, the organic components, though restricted in amount, will be the more important. On bits of plant residues and on soil humus accordingly there will congregate all kinds of soil microorganisms and macroorganisms and it is in this biologically

active area that the factors of antagonisms, antibiosis, and synergisms will come into play and be of the utmost importance in the survival of an organism. It is on these good particles that organisms will excrete their antibiotic substances and it is here that a high enough concentration may be built up to affect other more sensitive organisms. If we could separate from a soil sample these "microscopic food particles" and test them for antibiotic qualities, we might find that for some of them the antibiotic activity is very high. The more inert materials of the soil act as a diluent for some of the active antibiotic substances and may dilute, adsorb, or neutralize the effect of their lethal qualities, thus permitting many organisms to survive. Thus each microscopic food particle will be like an island of life of its own and the types of antagonistic organisms inhabiting it will determine to a large extent which of the pathogenic species will be able to survive there. If a plant pathogenic species is sensitive to a great variety of compounds and therefore to antibiotic products produced by many of the antagonists, then there is a good chance that most of the food particles it will encounter in a soil environment will have associated with them organisms that are detrimental to its existence (e.g. *E. tracheiphila*, *P. glycinea*, *P. phaseolicola*, *X. translucens*, etc.); while, on the other hand, pathogens that are not sensitive to many antagonists will encounter in the soil many food particles which will not contain any organisms detrimental to them and hence will display high survival rates as in the case of *E. carotovora*, *E. atroseptica*, and *P. marginata* for which very few antagonists were found.

Findings made under artificial conditions cannot be applied directly to such a highly dynamic system as the soil environment. There are in the soil possibilities of synergistic, competitive, parasitic, and toxic activities of each individual with all the others. In such complex systems one cannot evaluate the importance of single factors. We cannot, however, understand complex activities without analyzing, simplifying, and then resynthesizing, even though this may endanger the functional unity of the whole system (24, 45, 46). Although results obtained in culture with isolated organisms should not be interpreted as being applicable to soil conditions, it is highly improbable that those organisms which are capable of producing antibiotics in culture were not to do so in the soil (4). Thus an agar medium might be looked upon as being a very special type of a giant-sized food particle without the effect of inert diluents. Therefore some of the antagonisms exhibited in culture may be homologous to those exhibited in the soil but on a much simplified scale. If such an approach merely demonstrates certain trends that might occur in nature, it will have served its purpose.

Acknowledgments

I would like to express my appreciation and sincere gratitude to Prof. D. L. Bailey for his helpful interest, encouragement, and assistance throughout the course of this investigation. Thanks are also due to Dr. E. F. Palmer, Director of the Vineland Horticultural Experiment Station, for making available a summer student assistantship and the facilities of the Station; and

to the National Research Council for providing a Studentship during the 1951–52 term. The writer is also indebted to Dr. L. W. Koch, Officer-in-Charge of the Science Service Laboratory, Harrow, Ontario, for rendering available certain facilities for the completion of the work in its final stages.

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